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RESISTANCE IN MAIZE TO THE TROPICAL AMERICAN RUST FUNGUS, *PUCCINIA POLYSORA* UNDERW.

I. GENES Rpp_1 AND Rpp_2

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Received 18.i.57

THE tropical maize rust fungus, *Puccinia polysora* Underw., on its arrival in West Africa in 1949 (Rhind, Waterston and Deighton, 1952) and in East Africa in 1952 (Nattrass, 1953), encountered a susceptible maize population and caused severe losses. Because Cummins (1941) had shown that this species is widespread and long-established in Central America, it seemed possible that resistant maize might have evolved there ; and consequently we instituted a search for resistance genes in maize types imported from this source.

The work here described started late in 1952 as a contribution to a co-operative plant-breeding programme for controlling the disease, which has, subject to certain reservations, proved successful (Storey, Howland, Hemingway, Jameson, Baldwin, Thorpe and Dixon, *in press*). Resistance tests of seedlings in the Muguga glasshouses, and breeding from selected plants, had allowed us in 1953 not only to issue to the breeders maize lines carrying resistance genes but also to provide a broad outline of the genetic basis of resistance to guide their work. We continued breeding to complete the genetic analysis ; and, not unexpectedly, this has led to some modification of our early conclusions (Storey and Ryland, 1954).

In planning this research and interpreting the data, we received much help from discussions with Sir Joseph Hutchinson, F.R.S., and other colleagues. Experimental methods were guided at the start by the extensive work of pathologists and breeders on the rusts of small cereals and particularly by Dr R. M. Nattrass's experience with the rusts of wheat in East Africa.

1. EXPERIMENTAL METHODS

The general procedure was to test seedlings by inoculation with the rust fungus, to select resistant ones and to grow these to maturity and self-pollinate or cross. All stages were carried out in glasshouses, where work could continue throughout the year without dependence on season. The glasshouses were not temperature-controlled, except that heaters operated when temperatures fell below 70° F. Day length at Muguga is nearly uniform throughout the year. Technical details are as follows.

Plant culture. Seedlings were raised in soil blocks and those selected in tests transplanted to 4-gallon containers and grown on in a large glasshouse. Throughout

growth plants were watered with a dilute nutrient containing: N, 120/p.p.m. as ammonium sulphate; P_2O_5, 25/p.p.m. as superphosphate, and K, 15/p.p.m. as potassium sulphate. Insect infestations were controlled, without damage to the plants, by treating the soil with Schradan (55 to 66 per cent. A.I.) * at 3 ml. per container, at 6-weekly intervals if needed.

Inoculation. Second and third leaves of young seedlings were painted with 0·25 per cent. gelatine solution and fresh uredospores spread by spatula on the wet surface; or, in later work, a suspension of spores in the gelatine solution was spread by brush.

Infection. Immediately after inoculation, the group of seedlings was covered with a polythene † "hood" for 24 to 48 hours in a shaded glasshouse. (Infection often failed if direct sunlight fell on the plants.) For critical work with rust strains, each seedling was individually covered with a small polythene bag held open by an expanding metal collar pressed into the soil. The collars were heat-sterilised and only new polythene tube was used.

Incubation. After 24-48 hrs. seedlings were transferred to an unshaded or partly shaded glasshouse and hoods removed. Uredosori of the fungus began to appear on inoculated leaves of susceptible plants at 8-10 days from inoculation; and rust reactions were classified at 10, 14 and 18 days.

2. EXPERIMENTAL MATERIAL

The fungus. Two physiologic strains of *P. polysora* were available for this work:—

Race EA.1. Established in our glasshouses from uredospore collections made in 1952 in Zanzibar, Tanganyika and Uganda. At first these "local strains" were maintained in separate houses; and all early tests were replicated in two, and usually all three houses. No significant differences were observed in parallel tests and it is believed that this group constitutes a single physiologic race.

Race EA.2. Appeared spontaneously in the Muguga glasshouses in early 1955 and was recognised by a differential reaction in certain maize lines (Ryland and Storey, 1955). This race has not yet been observed in the field.

The two races have been maintained on maize in separate glasshouses, specially equipped to avoid accidental contamination, by inoculating simultaneously a differential host and a susceptible host. This differential was resistant to Race EA.1, and consequently, if it remained free from sori, the parallel susceptible plants were assumed to be carrying only this race; but the differential was susceptible to Race EA.2 and sori formed on it were necessarily this race.

The plants. We included in our tests 68 maize varieties and lines from African sources and 203 from Central and North America and the Caribbean area. Resistant plants were identified in 45 of the American lines; but the studies here reported are confined to three only, referred to under their "AFRO" accession numbers.

AFRO.24. Received from Dr W. R. Stanton, West African Maize Research Unit, Ibadan, Nigeria, as a first selected progeny from line "SLP 20-4A", from the Rockefeller Foundation, Mexico City.

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* As "Pestox III", supplied by Pest Control Ltd.

† "Alkathene" tubular film, supplied by Imperial Chemical Industries Ltd., grade 500 for the large hoods and grade 150 for the small tubes.

AFRO.27. Received from Dr Stanton as a first selected progeny from the cross, " LII 123-2-1-8 × Coah. 8-56A-4-2 ", from the Rockefeller Foundation, Mexico City.

AFRO.29. Variety " Colombia 2 ", received from Dr L. M. Roberts, The Rockefeller Foundation, Estacion Agricola Experimental, Medellin, Colombia.

Six susceptible African varieties were used from time to time as parents in crossing with resistant plants, and 8 as experimental controls.

3. THE CLASSIFICATION OF INFECTION TYPES

The following system of qualitative classification, adapted from that of the workers with stem rust of wheat (*e.g.* Stakman, Levine and Loegering, 1944), has been used.

Class " *O* ". No visible effect on the inoculated leaf.

Class " *O1* ". Visible chlorotic or necrotic lesions on the inoculated leaf, never developing uredosori during the observation period (plate, figs. 1 and 2).

Class " *1* ". Lesions starting as in Class " O1 " but necrotic early, enlarging and developing few or many small uredosori. Frequency of sori indicated by appended signs from " — — " to " + + " (plate, fig. 3).

Class " *4* ". Uredosori, beginning to open at 8-10 days, forming spores freely, later enlarging and sometimes expanding by rings of secondary sori. Necrosis absent in early stages ; chlorosis variable but typically no more than a barely visible paling of the leaf tissue, rarely a yellow diffuse " halo " around the sori (plate, figs. 5 and 6).

Class " *X* ". Intermediate between Classes " 1 " and " 4 ". Typically Class " 1 " lesions with small sori, mixed with larger sori on intervening green tissue (plate, fig. 4).

Class " O1 ", although based on the objective character of absence of sori, proved to be in part an unfortunate choice, for experience showed that two grades could be recognised. In Grade " (*a*) " the individual lesions were $\frac{1}{4}$ to $\frac{1}{2}$ mm. diameter, normally diffuse-edged and chlorotic (plate, fig. 1) but sometimes becoming necrotic and brown. In grade " (*b*) " lesions expanded to 1 mm. and often more in diameter and became necrotic early (plate, fig. 2). Grade " (*a*) " is normally distinctive ; Grade " (*b*) " differs in character from Class " 1 " only in lacking sori, and appears to be one end of a series passing through " 1 — — " to " 1 + + ".

In its typical form Class " X " is distinctive ; but we have been forced to use it for a range of intermediates between " 1 + + " and " 4 ". At the ends of this range differentiation has been difficult and uncertain.

4. RESULTS OF EXPERIMENTS

Early experiments were necessarily confined to inoculating maize with rust Race EA.1 ; and the reaction to this race was the criterion of selection for all the breeding described. Only after EA.2 had been isolated in 1955 was it possible to test sample maize lines against this race.

(i) *The susceptible reaction*

Sixty-eight African maize varieties, many of which were known to be susceptible in the field, reacted Class " 4 " in tests against EA.1. A sample of 15 of these, tested against EA.2, also reacted " 4 ". These variety tests included 1094 seedlings inoculated with EA.1 and 180 with EA.2. In addition, as experimental controls some ten thousand seedlings of East African varieties were inoculated with EA.1 and one thousand with EA.2. The only deviations from the characteristic Class " 4 " reactions were :—(1) a number classed " O ", due, we believe, to accidental failure of the inoculation (see below) ; and (2) a number showing exceptionally severe chlorosis.

Seven Class " 4 " plants, selected from families of American origin that had segregated into " O1 ", " 1 " and " 4 ", gave on selfing only Class " 4 " plants in 93 seedlings of the S1 families.

We conclude that a Class " 4 " plant does not carry a gene for resistance of the kind here studied. We are less certain whether the severely chlorotic " 4 " reaction may be genetically controlled. There are strong indications that chlorosis is often determined by the plant's nutrition and environment ; and experimental tests of families derived from chlorotic parents, under the limited environmental control available to us, gave no decisive evidence that chlorosis was inherited. In certain American maizes, however, we encountered a characteristic chlorotic reaction that is inherited. Our studies of this will be reported later.

(ii) *The resistant reactions*

Workers with wheat rusts interpret Class " O " as denoting immunity. We have not found this to be true of maize and *P. polysora*. Thus 16 families were raised by selfing Class " O " plants recorded in families otherwise susceptible ; all of 195 plants in the S1 families were classed " 4 ". Only when Class " O " plants were selected from families segregating " O1 ", " 1 " and " 4 " was there any exception ; two such S1 families themselves segregated " O1 ", " 1 " and " 4 ". We conclude that Class " O " in our experiments has denoted no more than accidental escape from infection ; and indeed, when methods had been improved by experience, very few plants were ever recorded " O " on both inoculated leaves.

For this reason we have excluded Class " O " plants from the records of our experiments.

The form of resistance that we have studied is that denoted by Classes " O1 ", " 1 " and " X ". In accordance with current thought on the rust fungi, we interpret these classes as indicating a hypersensitive reaction by the plant, causing the invading mycelium to be starved of nutrients and so prevented from developing to the sporing stage or hindered from producing more than small sori. We briefly reported our conclusion that this hypersensitive reaction is controlled by a single dominant major gene (Storey and Ryland, 1954) ; but

we now modify this by postulating two such genes as recognisable under conditions existing in East Africa to-day.

Resistance in AFRO.29. Seedlings of this variety, as received, reacted to *P. polysora*, Race EA.1, as follows :—

9—" O1 " : 5—" 1 " : 32—" 4 ".

In table 1 are assembled the results obtained in testing families derived from selected " O1 " and " 1 " plants. They are grouped in two main series, " I " and " II ", with sub-series " A " for selfed progenies and " B " for crosses with susceptibles.

In tests of Series I against rust Race EA.1 the S1 families from two selected " O1 "s segregated into " O1 " and " 4 " in proportions not significantly different from 3 : 1 ($P = 0{\cdot}1$). Selected " O1 "s from these families gave 4 S2 families that were all " O1 " ; and, since 3 S3 families from these were also classed " O1 " only, the families are considered to be homozygous for a resistance gene. Five S2 families segregated 3 : 1.

When " O1 " plants were crossed with susceptibles (Series IB), 3 F1 families segregated into 1 " O1 " : 1 " 4 ". The F2, by selfing " O1 "s from the F1, segregated 3 : 1 in all of 10 families. The F3 showed 8 families all classed " O1 " and 14 that segregated.

In all these tests the resistant class was " O1 ", Grade " (*a*) " ; except that 3 Class " 1 " seedlings were recorded in a total of 758 resistant ones. The " O1 "s varied within a narrow range, mainly showing only chlorotic lesions but, less frequently, similar sized lesions that were brown and necrotic. There was no evidence that homozygous plants were consistently different from those that were heterozygous. Our procedure, because our main purpose was breeding for practical control, was to select as parents for later generations those plants showing only the mildest chlorotic reaction ; nevertheless, the proportions of 4 homozygous to 5 segregating families in the S2 generation, and 8 to 14 in the F3, are not significantly different from the 1 : 2 ratio expected from a random selection of resistant parents within a family segregating 3 : 1.

When sample families from the above lines were tested against Race EA.2 they were recorded Class " 4 " ; their reactions were characteristic of susceptible controls, although there was a tendency for the sori to be smaller at first and to expand more slowly. One exception, which we cannot explain, was encountered in 2 F3 families from the cross. The F2 family from which both arose had segregated normally into " O1 " and " 4 " against EA.1 ; but both F3 families were recorded " O1 " against EA.1 and severe " 1 " against EA.2.

The evidence of Series I, on balance, therefore, indicates a single, fully dominant gene, conferring the power to react " O1 (*a*) " against Race EA.1 but failing to confer any resistance against EA.2. If the gene is subject to modification by minor genes—accounting possibly

TABLE 1

Tests of derivatives from AFRO.29 by (A) selfing, (B) crossing with susceptibles

Series	Generation	Group	Origin and rust classes of parents against EA.1	Totals in each rust class in tests against EA.1					Totals in each rust class in tests against EA.2			
				No. of families tested	Resistant		Susceptible	Segregation ratio postulated*	No. of families tested	Resistant		Susceptible
					"O1"	"1"	"4"			"O1"	"1"	"4"
IA	S1		AFRO.29 (" O1 ") selfed . .	2	41	0	7	3 : 1	...	...	...	...
	S2	(*a*)	Selected S1 (" O1 ") selfed . .	4	160	0	0	1 : 0	3	0	0	31
		(*b*)	Same	5	74	1	25	3 : 1	...	...	...	...
	S3		Selected S2 (*a*) (" O1 ") selfed .	3	42	0	0	1 : 0	1	0	0	8
IB	F1		AFRO.29 (" O1 ") × susceptible (" 4 ") and reciprocal	3	24	0	23	1 : 1	3	0	0	38
	F2		Selected F1 (" O1 ") selfed . .	10	108	0	42	3 : 1	2	0	0	20
	F3	(*a*)	Selected F2 (" O1 ") selfed . .	8	173	0	0	1 : 0	6	0	0	60
									2	0	31	0
		(*b*)	Same	14	133	2	38	3 : 1	2	0	0	14
IIA	S1		AFRO.29 (" 1 ") selfed . .	1	0	29	8	3 : 1	1	0	3	3
	S2	(*a*)	Selected S1 (" 1 ") selfed . .	1	1	11	0	1 : 0	1	3	8	0
		(*b*)	Same	1	1	5	2	3 : 1	1	0	3	2
IIB	F1		Susceptible (" 4 ") × AFRO.29 (" O1 ")	1	8	0	4	1 : 1	1	0	5	5
	F2		Selected F1 (" O1 ") selfed . .	3	29	0	8	3 : 1	2	9	14	3
	F3		Selected F2 (" O1 ") selfed . .	3	21	0	10	3 : 1	1	2	8	4

* Ratio expressed as—(Resistant Classes) : (Susceptible Class). The χ^2 tests have revealed no significant deviation from these ratios, either in the totals recorded above, or—with rare exceptions—in the individual numbers for each separate family. This is true also of all later tables where segregation ratios have been postulated.

for the variation in intensity of necrosis—this effect has been only slight in our trials. We designate this gene **Rpp$_1$**.*

Series II of table 1 is markedly different. The parent of the selfed lines was itself classed " 1 " against EA.1, and the resistant plants in its derivatives were predominantly " 1 ", both against EA.1 and also against EA.2. The same held generally for families derived from crosses with susceptibles, except that the resistant plants were all classed " O1 " against EA.1 (but mostly " 1 " against EA.2). Family ratios did not deviate significantly from those expected if a single dominant gene was concerned; but this gene is not **Rpp$_1$** because it is effective against Race EA.2. The gene is indistinguishable, on the limited evidence of this series of AFRO.29 derivatives, from the **Rpp$_2$** identified in AFRO.24.

Resistance in AFRO.24. The original AFRO.24 material proved on test to be all resistant to Race EA.1, reactions being classed " O1 " and " 1 ". Figures from testing derivatives from (A) selfs and (B) crosses are presented in table 2, in the same general form as that used in table 1.

In the selfed lines, one S1 family was entirely resistant to EA.1 (Classes " O1 " and " 1 "), and 3 S2 families in this line were also resistant. Nine S1 families segregated 3 resistants to 1 susceptible; and, of the S2 families raised from selected resistants from the segregating S1 families, 6 were homozygous and 6 segregated. Four of the homozygous families, tested against Race EA.2, were entirely resistant (" O1 " and " 1 ").

The derivatives from crosses with susceptibles behaved generally in accordance with expectation. One F1 family contained only resistant plants, and the other segregated 1 : 1. All the 17 F2 families segregated 3 : 1. Of the F3 families, 23 were presumed homozygous and 8 segregated. Six of the homozygous families, tested against EA.2, were recorded " O1 " and " 1 " only.

The gene from AFRO.24 differs from the **Rpp$_1$** of AFRO.29, not only in conferring equal resistance to the two rust races, but in other respects also. Whereas plants carrying **Rpp$_1$** reacted against EA.1 in the Class " O1 (*a*) " with minor variations, derivatives of AFRO.24 might show the whole range from " O1 (*b*) " to " 1++ ". This wide range can be attributed in part, but only in part, to environmental differences encountered in experiments done at different seasons. To eliminate this environmental effect, and to obtain more exact figures for the several grades of Class " 1 ", we repeated tests of certain families, against EA.1 only, all inoculations being done during 5 days in June 1956 (table 3). On this occasion homozygous lines in the S1 and S2 showed only " O1 (*b*) " phenotypes; while segregating selfed lines showed the range from " O1 (*b*) " to " 1+ " and " 4 ". Among the crosses, two homozygous F3 families (last line of the

* This designation has been adopted on the advice of Dr M. M. Rhoades, Secretary to the Maize Genetics Co-operation, the University of Illinois, U.S.A.

TABLE 2

Tests of derivatives from AFRO.24, by (A) selfing, (B) crossing with susceptibles

Series	Generation	Group	Origin and rust classes of parents against EA.1	Totals in each rust class in tests against EA.1					Totals in each rust class in tests against EA.2			
				No. of families tested	Resistant		Susceptible	Segregation ratio postulated*	No. of families tested	Resistant		Susceptible
					"O1"	"1"	"4"			"O1"	"1"	"4"
A	S1	(*a*)	AFRO.24 ("O1") selfed	1	23	12	0	1 : 0	...	...	...	...
		(*b*)	AFRO.24 ("O1"-"1") selfed	9	53	50	30	3 : 1	...	...	...	...
	S2	(*a*)	Selected S1 (*a*) ("O1"-"1") selfed	3	36	24	0	1 : 0	2	22	4	0
		(*b*)	Selected S1 (*b*) ("O1"-"1") selfed	6	117	27	0	1 : 0	2	22	3	0
		(*c*)	Same	6	38	21	20	3 : 1	...	...	...	...
B	F1	(*a*)	Susceptible ("4") × AFRO.24 ("O1")	1	0	35	0	1 : 0 (heterozygous)	1	0	6	0
		(*b*)	Same	1	0	6	8	1 : 1	...	...	...	...
	F2		Selected F1 (*a*) and (*b*) ("1") selfed	17	13	140	60	3 : 1	...	...	...	...
	F3	(*a*)	Selected F2 ("O1"-"1") selfed	23	70	440	0	1 : 0	5	8	24	0
		(*b*)	Same	8	6	55	25	3 : 1	...	...	...	...

* See note on table 1.

table) were all " O1 (*b*) " or " 1 — — ", whereas known heterozygous resistants in the F1 were " 1+ " or " 1++ ". Resistant plants in segregating families in the F2 and F3 generations covered the full range from " O1 (*b*) " to " 1++ ". We interpret this evidence as indicating that the gene is incompletely dominant.

Support to this hypothesis is given by the proportions of homozygous to segregating families realised in the S2 generation and the F3 from crosses (table 2). The 6 : 6 proportion in the S2 is not significantly different from the 1 : 2 ratio expected from random selection of parents in a family segregating 3 : 1 ; but 23 : 8, in the

TABLE 3

Second-series tests of certain families derived from AFRO.24
(Inoculated with EA.1 only in June 1956)

Series	Nature of families	Segregation ratios in first tests	No. of families tested	Totals in each rust class					
				O1(*b*)	1— —	1+ —	1++	4	
A	S1 and S2 AFRO.24	1 : 0	2	52	0	0	0	0	0
	Same	3 : 1	5	10	11	4	5	0	14
B	F1—Suscept. × AFRO.24	1 : 0 (heterozygous)	1	0	0	0	0	0	0
	Same	1 : 1	1	0	0	0	0	4	5
	F2 and F3 from cross	3 : 1	6	8	13	14	9	1	6
	F3 of cross	1 : 0	2	8	12	0	0	0	0

F3, is very markedly so. In fact, we had selected as parents those plants showing the least severe reactions—Class " O1 " preferably, or the mildest " 1 ". This selection appears to have resulted in the propagation of a disproportionate number of homozygotes, indicating again that the gene is incompletely dominant.

Nevertheless, it is evidently not possible to select genotypes with certainty by inspection ; there is no more than a tendency for the selection we have practised to give homozygotes. It seems necessary to postulate some modification by minor genes, causing the reactions of each genotype often to overlap two or more sub-classes.

We designate the major gene from AFRO.24 as the " type " of **Rpp**$_2$.

Resistance in AFRO.27. The original imported material, tested against rust Race EA.1, was recorded :—

1—" O1 " : 12—" 1 " : 20—" 4 ".

Records from testing selfed and out-crossed derivatives from selected " O1 " and " 1 " parents are recorded in table 4.

Results obtained are closely similar to those from derivatives of AFRO.24, except that throughout there is a tendency for reactions

TABLE 4

Tests of derivatives from AFRO.27, by (A) selfing, (B) crossing with susceptibles

Series	Generation	Group	Origin and rust classes of parents against EA.1	Totals in each rust class in tests against EA.1					Totals in each rust class in tests against EA.2			
				No. of families tested	Resistant		Susceptible	Segregation ratio postulated*	No. of families tested	Resistant		Susceptible
					"O1"	"1"-"X"	"4"			"O1"	"1"-"X"	"4"
A	S1	(*a*)	AFRO.27 (" 1 ") selfed . .	2	28	63	0	1 : 0	2	6	6	0
		(*b*)	AFRO.27 (" O1 "-" 1 ") selfed .	4	5	35	21	3 : 1	...	...	...	...
	S2	(*a*)	Selected S1 (*a*) (" O1 "-" 1 ") sibbed	1	8	36	0	1 : 0	1	0	10	0
		(*b*)	Selected S1 (*b*) (" O1 "-" 1 ") selfed	5	7	105	0	1 : 0	5	12	44	0
		(*c*)	Same	8	0	97	28	3 : 1	...	...	...	...
B	F1		AFRO.27 (" 1 ") × susceptible (" 4 ") and reciprocal	2	0	12	24	1 : 1	...	...	...	...
	F2		Selected F1 (" 1 ") selfed . .	3	0	27	7	3 : 1	...	...	...	...
	F3	(*a*)	Selected F2 (" 1 ") selfed . .	2	0	43	0	1 : 0	2	9	32	0
		(*b*)	Same	2	0	14	8	3 : 1	...	...	...	...

* See note on table 1.

to be more severe. Indeed, the range extended beyond that characteristic of " 1++ " into the rather indeterminate Class " X " ; because, however, our early records are insufficiently detailed, we have grouped the " 1 " and " X " reactions in table 4.

If we combine " O1 ", " 1 " and " X " as constituting together a resistant group, the segregation ratios realised point to a single dominant, or partly dominant, major gene. In tests against EA.1, 2 S1 families —and an S2 from one of these—were wholly resistant ; and segregating families in the selfed lines gave proportions not significantly different from a 3 : 1 ratio. Among the crosses with susceptibles, the F1 segregated 1 : 1 (but barely significantly), the F2 3 : 1, and, in the F3, 2 families were homozygous and 2 segregated.

TABLE 5

Second-series tests of certain families derived from AFRO.27
(Inoculated with EA.1 only in May 1956)

Series	Nature of families	Segregation ratios in first tests	No. of families tested	Totals in each rust class			
				" O1 "	" 1 "	" X "	" 4 "
A	S1 of AFRO.27	1 : 0	2	0	94	2	0
	S1 and S2 from AFRO.27 .	3 : 1	8	0	24	35	14
B	F1—Susceptible " × " AFRO.27	1 : 1	2	0	0	5	9
	F2 and F3—Selfs . .	3 : 1	4	0	11	14	11
	F3—Self	1 : 0	1	0	14	0	0

Ten selected homozygous families were tested also against Race EA.2 ; all were recorded " O1 " and " 1-X ".

The proportions of homozygous to segregating families in the S2 and F3 generations (7 : 10, in total) did not indicate that in selecting their parents we had obtained a significant excess of homozygotes. Nevertheless, as with AFRO.24, we repeated tests against EA.1 of certain families during May 1956 (table 5). In this series, Class " O1 " was not encountered. Believed homozygous families were predominantly Class " 1 " (108 " 1 "s to 2 " X "s). Two F1 families from crosses, where the resistant plants were necessarily heterozygous, gave only " X " resistants. In segregating families the resistant plants were distributed in Classes " 1 " and " X ". There is, therefore, a suggestion of incomplete dominance.

Thus in AFRO.27 we appear again to have identified an incompletely dominant gene conferring equal resistance to rust Races EA.1 and EA.2. Although the resistant reaction in homozygotes is not so strong as when **Rpp$_2$** from AFRO.24 is present and the grade of the heterozygote is lower, we do not feel justified, in the absence of more conclusive evidence, in postulating for AFRO.27 a major gene different from **Rpp$_2$**.

5. DISCUSSION

It is hardly open to question that the genes we have designated $\mathbf{Rpp_1}$ and $\mathbf{Rpp_2}$ are not identical. The primary difference lies in the differential reactions against the two physiologic races of *P. polysora* existing in East Africa : $\mathbf{Rpp_1}$ confers high resistance against EA.1, but none against EA.2, while $\mathbf{Rpp_2}$ confers equal resistance against both races. Furthermore the "O1 (*a*)" reaction of $\mathbf{Rpp_1}$ is normally distinguishable by inspection from the "O1 (*b*)" of $\mathbf{Rpp_2}$, the highest grade of resistance this gene is capable of conferring. $\mathbf{Rpp_1}$ appears to be completely dominant, and its resistant reactions are only slightly, if at all, modified by minor genes.

On the other hand, $\mathbf{Rpp_2}$, as identified in AFRO.24, besides showing no differential reaction against the two rust races, is incompletely dominant, and the range of the resistant reactions seems most reasonably explicable as minor-gene modification.

Whether we are correct in suggesting that the gene obtained from AFRO.27 (and from AFRO.29 in part) is identical with that from AFRO.24 is uncertain. The AFRO.27 gene resembles it in being equally resistant to both races of the rust, in being incompletely dominant and in being apparently subject to considerable minor-gene modification. That the AFRO.27 derivatives show more severe reactions than those from AFRO.24 might be due to differences in the minor-gene complements of these two lines. It is possible that new physiologic races may appear in East Africa against which the genes from these two sources may react differently ; but meanwhile the preferable course is to regard them as identical.

The expectation that maize resistant to *P. polysora* would have evolved in the home of this fungus has been realised, as Stanton and Cammack (1953) have reported on other evidence. It is surprising that, of the 203 separate samples of maize received from American sources, not one was homozygous for a resistance gene. At first sight it appears that selection pressure at source has not been as great as might have been expected from the severe effects of the disease in Africa. But it must be remembered that in East Africa we have studied the genes conferring resistance to only two races of *P. polysora* ; and it is possible that in Central America maize has evolved under infection by a range of physiologic races largely different from those with which we have worked.

6. SUMMARY

1. When *Puccinia polysora* appeared in Africa, it encountered a susceptible maize population ; but resistance of the hypersensitive type was found in certain maize lines introduced from Central America. The genetics of resistance were studied by controlled inoculation of seedlings in glasshouses and by breeding from individual plants selected on seedling tests.

Plate

Portions of leaves from maize seedlings inoculated with *Puccinia polysora* Underw.
All ×4·5.

FIG. 1.—Class "O1", Grade "(*a*)", at 18 days. Typical reaction of plant carrying Rpp_1; the lesions are yellow, slightly shrunken and remain small.

FIG. 2.—Class "O1", Grade "(*b*)", at 18 days. A rather severe form of the reaction in a plant carrying Rpp_2. The lesions are necrotic and pale brown. (Only half the leaf was photographed.)

FIG. 3.—Class "1++", at 18 days. Typical severe reaction in plant carrying Rpp_3. Lesions pale brown, with small open sori.

FIG. 4.—Class "X", at 18 days. Lesions similar to those of "1++", together with some larger sori (mainly on left-hand side of photograph), erupting from leaf tissue only slightly chlorotic.

FIG. 5.—The susceptible reaction, Class "4", at 10 days. Sori just erupting from tissue only faintly chlorotic.

FIG. 6.—Class "4" at 20 days. The sori have enlarged greatly and extruded large masses of uredospores. Chlorosis still confined to faint yellowing of the leaf tissue.

FIG. 1

FIG. 2

FIG. 3

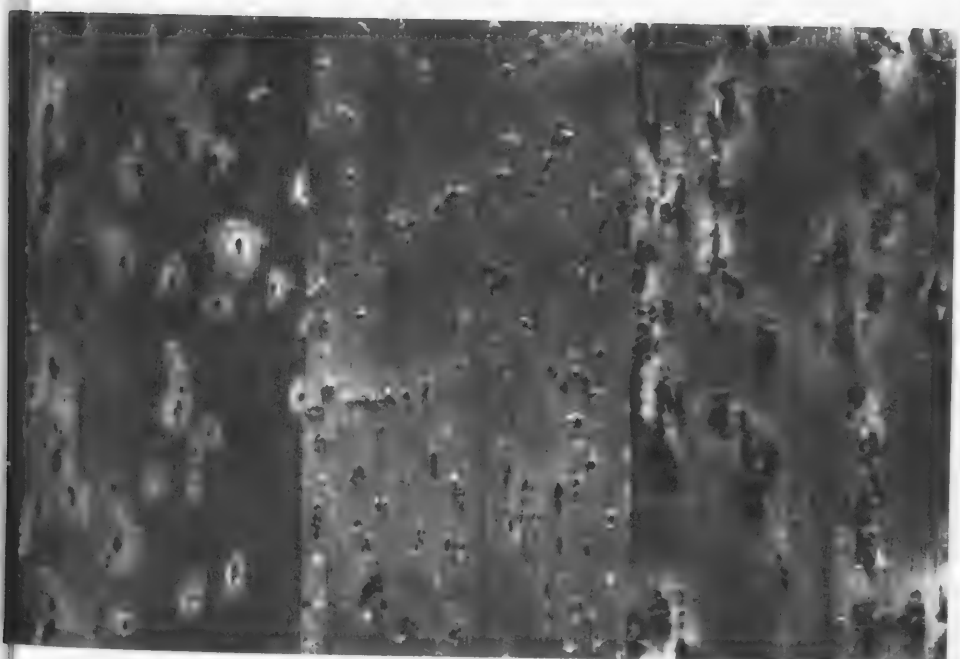

FIG. 4

FIG. 5

FIG. 6

2. Two major genes for resistance were recognised. The first, $\mathbf{Rpp_1}$, conferred high hypersensitivity against the prevalent physiologic race of *P. polysora* (Race EA.1), made evident on inoculated leaves by minute chlorotic-necrotic lesions without uredosori ; but it conferred no recognisable resistance to a second race of the fungus (EA.2). $\mathbf{Rpp_1}$ appeared to be fully dominant. The second gene, $\mathbf{Rpp_2}$, conferred a lesser hypersensitivity, typically necrotic lesions with small sori, but was equally effective against Races EA.1 and EA.2. This gene was incompletely dominant. $\mathbf{Rpp_2}$ appeared to be subject to considerable minor-gene modification, but $\mathbf{Rpp_1}$ much less so.

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STUDIES ON *STREPTOCARPUS*

III. GENETICS AND CHEMISTRY OF FLOWER COLOUR IN THE GARDEN FORMS, SPECIES AND HYBRIDS

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1. INTRODUCTION

In a previous paper (Lawrence *et al.*, 1939) a first account † was given of the genetics and chemistry of flower colour in the garden forms of *Streptocarpus* ($2n = 32$). This paper deals mainly with aspects of gene action and interaction in pigment production, the latter being of special interest.

2. CHEMISTRY OF FLOWER COLOUR

The structural formulæ of the anthocyanidin (sugar-free) pigments relevant to *Streptocarpus* are given in fig. 1. Peonidin is described as a derivative (by partial methylation) of cyanidin and similarly petunidin and malvidin are derivatives of delphinidin.

Anthocyanidins do not occur free in nature but in combination with one or more molecules of a sugar, the compound being an anthocyanin. One molecule of sugar is always situated at position 3- (monose). If a second one is present, it may either be attached to the first one (biose) or linked with the anthocyanidin at position 5- (dimonoside) ; for glycoside terminology see Robinson and Robinson, 1932. In *Streptocarpus* flowers the anthocyanidin glycosides are of three kinds : (i) 3-monoside, having one hexose molecule at position 3- on the pigment molecule, (ii) 3-pentoseglycoside, having both a hexose and a pentose molecule at position 3- and (iii) 3 : 5-dimonoside, having one hexose molecule at position 3- and another at position 5-, fig. 2.‡

Another group of sap-soluble pigments, ranging in colour from near white to yellow, is conveniently denoted by the term " anthoxanthin " (flavones, flavonols, flavanones) and to avoid circumlocation we shall include chlorogenic acid under this heading. Some anthoxanthins have the property of making some anthocyanins bluer and these are described as co-pigments (Robinson and Robinson, 1931).

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† The work in progress was suddenly broken-up by the outbreak of the 1939-45 war, at the point where a general survey had been completed but the more intensive investigations had barely begun. Post-war changes in the authors responsibilities have delayed analysis and publication of results.

‡ Recent findings (p. 324) suggest that two types of glycoside with sugars in both 3- and 5- positions are present, but the term 3 : 5-dimonoside is retained throughout this paper pending further work on these glycosides.

Two nearly colourless anthoxanthins, one of them a co-pigment, occur in *Streptocarpus* flowers.

3. GENETICS OF GARDEN FORMS

(i) *Anthocyanins*

The garden forms of *Streptocarpus* originated 70 years ago, from crosses between *S. dunnii* Mast., *S. rexii* Lind., and *S. parviflorus* Hook, made by W. Watson at the Royal Botanic Gardens, Kew. Selection by nurserymen during the next twenty years led to the establishment of the garden forms much as we know them to-day. For all practical purposes they may be regarded as forms of *S. rexii* into which have been bred three major flower colour genes from *S. dunnii*, fig. 2. Thus,

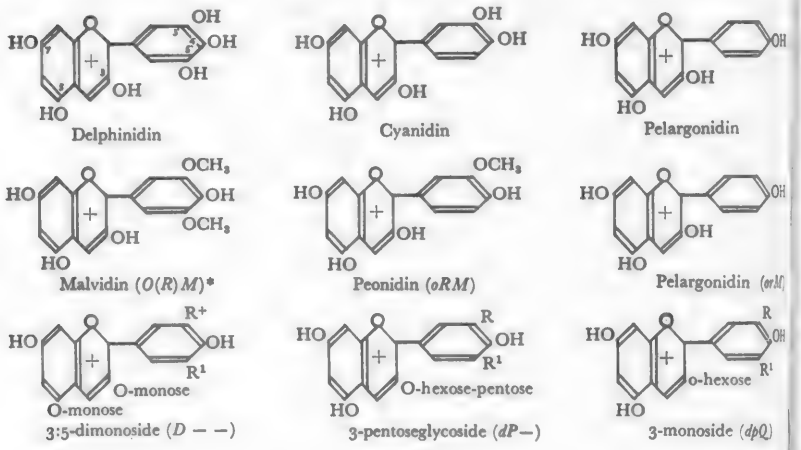


FIG. 1.

Structural formulæ for flower pigments relevant to *Streptocarpus*.
Upper line : the three main naturally occurring anthocyanidins.
Middle line : the three main anthocyanidins in *Streptocarpus*.
Lower line : three glycosides in *Streptocarpus*.
Any one of the glycosides may be associated with any one of the anthocyanidins (middle line).

* petunidin, 3′-methyl delphinidin, is sometimes found associated with malvidin, but is not considered in this paper.
+ where R and R^1 may be H, OH, or OCH_3.

although disturbed ratios may occur in breeding experiments with garden forms, their interspecific hybridity is not otherwise obvious.

Four main pairs of genes controlling flower colour in *Streptocarpus* have been identified previously (Lawrence *et al.*, 1939). Their phenotypic effects are as follows :—

V, necessary for general production of anthocyanin in leaves and flower stems.

F (previously *A*), with *V*, necessary for the general production of anthocyanidin in the flowers. In the presence of *r* and *o* (see below), the anthocyanidin is pelargonidin. Incompletely dominant.

R, substitutes a hydroxyl at position 3′- giving cyanidin in place of pelargonidin. Dominant.

O, epistatic to *R*. Substitutes two hydroxyls, one at position 3′- and one at 5′-, giving delphinidin in place of pelargonidin (or cyanidin). Dominant.

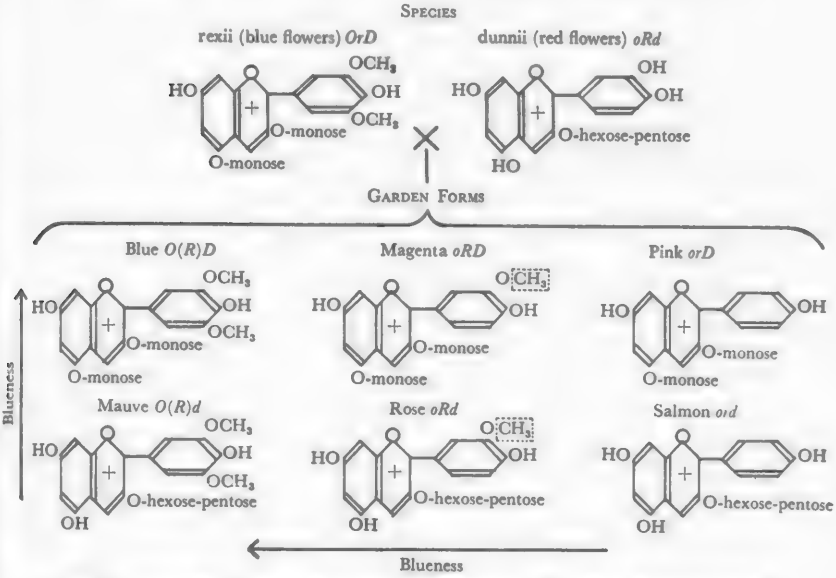


Fig. 2.—Chemical and genetical scheme for the inheritance of the main anthocyanins in the garden forms of *Streptocarpus*, showing their origin from artificial species hybridisation. The white-flowered *Streptocarpus parviflorus* was a third parent in this hybridisation and has the genetic constitution *ORD*.

D, produces 3 : 5-dimonoside in place of mixtures of 3-pentose-glycoside and 3 : 5-dimonoside (*d*) or 3-monoside and 3 : 5-dimonoside. Dominant.

Different combinations of these five pairs of genes give seven distinct classes for flower colour, *viz.* :—blue (*AORD* and *AOrD*), mauve (*AORd* and *AOrd*), magenta (*AoRD*), rose (*AoRd*), pink (*AorD*) and salmon (*Aord*). Fig. 2 summarises the origin and main genotypes and pigments of the garden forms.

The existence of *V* became known when a wild, white-flowered variant of *rexii*, viz. *rexii FB*, completely devoid of anthocyanin in flowers, flower stems and leaves, was introduced into the experiments.

White-flowered garden forms in our material usually have some

anthocyanin in the leaves and invariably in the flower stems which, in general, are conspicuously pigmented. On crossing *rexii FB* with

TABLE 1

Showing interaction of genes V *and* F *in floral pigmentation*

Family	White-flowered parents	Genotype	Cyanic peduncles		Acyanic peduncles	
			Flower colour		Flower colour	
			pale blue	white	pale blue	white
			VFi	*Vfi*	*vFi*	*vfi*
16/55	*rexii FB* selfed . .	*vvFFii*	0	0	0	50
13/34	1/33 selfed . . .	*VVffii*	0	128	0	0
3/55	1/33 × *rexii FB* . .	...	80	0	0	0
14/55	3/55 selfed . . .	*VvFfii*	57	10	13	
	Calculated 9 : 3 : 4 ratio		45	15	20	

appropriate garden forms, the progenies revealed that two complementary genes, *V* and *F*, were required to account for the inheritance of anthocyanidin in the flowers (table 1). All forms carrying

TABLE 2

Some characteristic phenotypes and their genotypes

Phenotype (anthocyanin)		Genotype
Flower stems (also calyxes and leaves in varying degree)	Corollas	
No pigment (green)	Pure white, or very faintly tinged	*rexii FB* :— *vFi*
		Garden forms :—
Pigment (red)	Pure white	*Vfi*
Pigment (red)	Pale anthocyanin	*VFi*
Pigment (red)	Medium to deep anthocyanin	*VFI*

V are identifiable on sight by their pigmented flower stems. A third gene *I* intensifies the anthocyanidin produced in the flowers by *V* and *F*. Some characteristic phenotypes and their genotypes are given in table 2 and the separate inheritance of *V*, *F* and *I* is summarised in table 3.

Re-examination of the older records of anthocyanin intensity in the garden forms showed that of the many hundreds of families recorded, only a few had distinct intensity classes and to these the *VFI* scheme can be fitted (table 4). The action of *I* can be identified

with certainty only in families uniformly homozygous or heterozygous for *F* (table 4) because, in most cases *F* and *I* are each cumulative in

TABLE 3

Inheritance of Vv, Ff *and* Ii

Cross or self	No. of families	Progeny *X* : *x*	χ^2		Degree of freedom	*P*
			Deviation from expectation	Hetero-geneity		
		1 : *1*				
Vv×*vv* . .	5	93 : 94	0·005	...	1	0·95
				12·779	4	0·02-0·01
Ff×*ff* . .	2	44 : 61	2·752	...	1	0·1-0·05
				0·896	1	0·5-0·10
Ii×*ii* . .	3	72 : 95	3·168	...	1	0·10-0·05
				0·863	2	0·90-0·50
		1 : *1* : *2*				
VvIi×*vvii* . .	6	54 : 52 : 105	0·043	...	2	0·98-0·95
				8·981	10	0·90-0·50
		3 : *3* : *2*				
Ffii×*FfIi* . .	2	31 : 28 : 18	0·284	...	1	0·90-0·50
				0·264	3	0·98-0·95
		9 : *7*				
VvFfii selfed .	3	68 : 45	0·708	...	1	0·90-0·50
				1·023	2	0·90-0·50
		9 : *3* : *4*				
VvIi selfed . .	3	64 : 29 : 18	6·686	...	2	0·05-0·02
				0·782	4	0·95-0·90
		3 : *1*				
Vv selfed . .	1	31 : 14	0·896	...	1	0·50-0·10

TABLE 4

Inheritance of Ii

Family	Parents	Anthocyanin intensity			
		Very deep	Deep	Medium	Pale
2/34	3/33 (*FFIi*) selfed . .	14	62	40	0
5/34	2/33 (*FFII*) selfed . .	99	0	0	0
8/34	2/33 (*FFII*) × 3/33 (*FFIi*) .	47		0	0
14/34	1/33 (*ffii*) × 2/33 (*FFIi*) .	0	109		0
6/34	3/33 (*FFIi*) × 1/33 (*ffii*) .	0	0	45	54
13/35	8^{3}/34 (*FFIi*) selfed . .	0	23	58	23
84/36	13^{15}/35 (*FFIi*) selfed . .	77	24	0	0
102/38	16/34 (*FFIi*) × 1/33 (*ffii*) .	0	48	45	0

action and dosage effects prevent the distinguishing of *VFI* from *VFi* genotypes.

Anthocyanin intensity in typical *S. rexii*, the pale-blue-flowered ancestral parent of the garden forms, is practically identical with that of *VFi* garden genotypes, whereas the deeper intensity of *S.*

dunnii, another ancestral parent, is similar to that of *VFI* forms. This suggests that just as the genes *O* and *D* were contributed by *rexii* and *R* by *dunnii*, so *i* is derived from *rexii* and *I* from *dunnii*.

It can now be seen that, whereas *V*, *F* and *I* are concerned with the general production of anthocyanin in the flowers, *O*, *R* and *D* modify the structure of the anthocyanin molecule and in this sense may be said to be specific. The terms "general" and "specific" have been used by Scott-Moncrieff (1939) in a special genetic sense.

TABLE 5

Flower colour	Genotypes tested (all *VVFF*)	Anthocyanins
Blue	*OORRDd* *OoRRDd* *OoRrDd*	Malvidin 3 : 5-dimonoside. Plus, usually, traces of anthocyanin giving Fe+ reaction.*
Magenta	*ooRRDd* *ooRrDD* *ooRrDd*	Peonidin 3 : 5-dimonoside. Plus varying amounts of cyanidin 3 : 5-dimonoside.
Pink	*oorrDD* *oorrDd*	Pelargonidin diglycoside. Plus traces of cyanidin diglycoside.
Mauve	*OORRdd* *OoRRdd* *OoRrdd* *OOrrdd* *Oorrdd*	Malvidin 3-pentoseglycoside (or 3-monoside †). Plus trace of Fe+ anthocyanin, plus malvidin 3 : 5-dimonoside containing slight traces of Fe+ anthocyanin.
Rose	*ooRRdd* *ooRrdd*	Peonidin 3-pentoseglycoside (or 3-monoside †). Plus some cyanidin 3-pentoseglycoside (or 3-monoside), plus peonidin 3 : 5-dimonoside, plus trace of cyanidin 3 : 5-dimonoside.
Salmon	*oorrdd*	Pelargonidin 3-pentoseglycoside (or 3-monoside †), plus pelargonidin diglycoside. Traces of cyanidin glycosides are often present.

* Fe+ refers to the positive ferric reaction given by delphinidin, petunidin and cyanidin, *i.e.* pigments containing an *o*- dihydroxyl group.

† Rarely, a little monoside may accompany pentoseglycoside or vice versa.

In this paper their usage is purely descriptive: "general" refers to pigment production without regard to the actual type produced and "specific" to a definite modification of the pigment molecule. The anthocyanins identified in seventeen different garden genotypes tested are listed in table 5.

(II) *Anthoxanthins*

An ivory-white anthoxanthin has been found to act as a co-pigment and can therefore be recognised visually in cyanic flowers by its blueing action. On a visual assessment the inheritance of the co-pigment is controlled by a single gene (*C*) (table 6). Chemical tests show that *cc* individuals sometimes contain a little pigment; *C* is

concerned, along with *O*, *R* and *D*, in specific flower pigmentation. Chromatographic tests showed that apigenin glycosides (fig. 3) are the only flavones present in quantity in flowers containing co-pigment i.e. *C* flowers. Since apigenin glycosides are known to be co-pigments in other genera, we may assume that they have the same function in *Streptocarpus*.

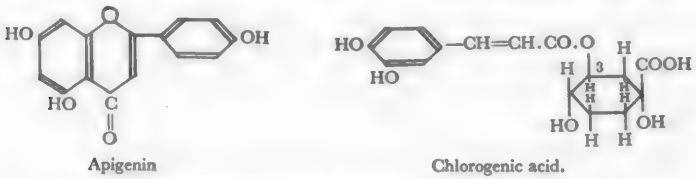
TABLE 6

Inheritance of the co-pigment (C)

Genotypes	No. of families	Observed	
		C	*c*
CC selfed . . .	1	80	0
Cc selfed . . .	1	60	23
cc selfed . . .	8	0	246
Cc × *cc*	7	122	118
cc × *Cc*	2	34	40
cc × *cc*	3	0	109

Pooled B1 data. $\chi^2 = 0{\cdot}006$, 1 D.F. $P = 0{\cdot}95-0{\cdot}90$
$\chi^2 = 2{\cdot}89$, 8 D.F. $P = 0{\cdot}95-0{\cdot}90$

A second anthoxanthin is found in all the many garden forms tested. It is not a co-pigment. This was proved by extracting the anthoxanthins from two sibs, a blue-mauve (*C*) and a red-mauve (*c*) and adding each anthoxanthin to the anthoxanthin-free anthocyanin



Apigenin Chlorogenic acid.

FIG. 3.—Structural formulæ for apigenin (co-pigment) and chlorogenic acid (non-co-pigment).

solutions from the same blue- and red-mauve plants. The anthoxanthin from the blue-mauve (*C*) strongly co-pigmented the anthocyanin from the red-mauve (*c*) whereas the anthoxanthin from the red-mauve did not co-pigment the anthocyanin from the blue-mauve plant. From analysis, using partition chromatography, the non-co-pigment appears to be chlorogenic acid (fig. 2). In passing, it may be noted that the constitution of *rexii* for co-pigment is *CC* and *dunnii cc*.

(iii) *Methylation*

In *Streptocarpus* and other genera, the 3′- and 5′-hydroxyl groups of the anthocyanidins may or may not be methylated, that is, have the hydrogen atom of the hydroxyl group replaced by a methyl group

(fig. 1). Methylation of the 4′-hydroxyl is not found in nature, therefore, all pelargonidin forms are unmethylated.

As will be seen from fig. 2, the anthocyanin in *S. rexii* (*Or*), one of the parents of the garden forms, is methylated, whereas in the other parent, *S. dunnii* (*oR*) it is not, *i.e.* gene *O* in *rexii* produces malvidin and gene *R* in *dunnii* cyanidin (*or* forms contain pelargonidin). When, however, we examined the garden forms, *R* was found to give, not cyanidin, as in *dunnii* but the methylated analogue, peonidin. Further, crossing a garden rose (*oR*, peonidin) or salmon (*or*, pelargonidin) to *dunnii* (*oR*, cyanidin) gave peonidin in the F_1, not cyanidin. Thus, *R* gives unmethylated pigment in *dunnii* but methylated pigment in the garden forms. The simplest explanation of this situation is that

TABLE 7

Inheritance of methylation

Genotype	*O*						*o*					
Percentage methylation class means :	100	87·5	62·5	37·5	12·5	0	100	87·5	62·5	37·5	12·5	0
1/37 selfed .	5	2	7	2	1	0	0	0	0	0	0	2
1/37 × *dunnii* .	5	2	4	2	1	1	1	5	5	4	4	15
2/37 × *dunnii* .	11	12	7	2	2	3	11	2	2	2	2	7
B1 totals .	16	14	11	4	3	4	12	7	7	6	6	22

nearly all garden forms are homozygous for a dominant gene for methylation. In fact out of 133 individuals tested, only two were unmethylated. *S. dunnii* may, therefore, be tentatively designated *oRm*, and the above mentioned garden forms *oRM*, where *M* indicates a gene or genes for methylation.

Some evidence on the inheritance of methylation has been obtained from derivatives of *rexii* × *dunnii* (1/37) and *rexii L* (a wild form related to *rexii*) × *dunnii* (2/37). *rexii* and *dunnii* are widely different species and the fertility of F_1 (completely methylated) and B_1 crosses is greatly reduced, *e.g.* one capsule of *rexii* normally produces some 2000 seeds, whereas the corresponding figure for the F_1 and B_1 capsules is of the order 10 and 100 respectively. Because of the high mortality of gametes or zygotes consistent segregation, in different families, of methylated and unmethylated progeny in terms of Mendelian ratios could hardly be expected. The results of the above crosses are given in table 7, the progeny being divided into six arbitrary classes for degree of methylation and distinguishing between *O* and *o* genotypes.

As will be seen, in both F_2 and B_1 (and in both *O* and *o* genotypes) completely unmethylated forms are at once recovered, and in B_1 totals the proportions of completely methylated and unmethylated progeny are sufficiently high as to suggest that a simple genetic system governs the inheritance of methylation.

(iv) *Glycosidation*

In discussing glycosides, we shall use the terms "primary" and "secondary" to distinguish dimonoside produced in *D* forms from that produced in *d*.

(a) *Primary dimonoside.* *D* produces 3 : 5-dimonoside and, on visual assessment of flower colour, we had previously reported this gene to be completely dominant. Chemical assessment of flower pigment now shows that its dominance depends in part on the presence of *O* and *R* (see table 12). Out of 25 *Dd* forms tested, twenty contained 100 per cent. dimonoside and five 98, 90, 90, 85 and 80 per cent. respectively.

(b) *Secondary dimonoside.* In the absence of *D*, the anthocyanin generally consists of a mixture of either 3-pentoseglycoside or 3-monoside with 3 : 5-dimonoside. In these mixtures, the proportion of dimonoside in garden forms varies more or less continuously from 20 to 95 per cent. Selection of types with high or low dimonoside content yielded forms that were largely or altogether true breeding for high or low dimonoside. Data which could be clearly interpreted were obtained by crossing selected garden forms to the two true breeding species, *dunnii* and *rexii*, which were also crossed with one another. The general position may be stated as follows :—

1. certain *dd* garden forms when selfed breed true for low dimonoside ;
2. certain low dimonoside varieties when crossed give only low dimonoside forms in F_1 ;
3. *S. dunnii* breeds true for low dimonoside ;
4. certain low dimonoside varieties crossed to *dunnii* give only *high* dimonoside forms in F_1 ;
5. certain garden forms breed true for high dimonoside.

These results can be explained by postulating two pairs of complementary genes responsible for secondary dimonoside production in *dd* genotypes, the low dimonoside garden varieties being *XXzz* in constitution, *dunnii* (low) *xxZZ* and the F_1's (high) *XxZz*. If this postulation is correct, it should be possible to get low dimonoside (*xxZZ*) forms from backcrossing the high dimonoside F_1's to *dunnii* and these should then give *low* dimonoside progeny in B_2 with *dunnii*. This was found to be the case and the results are given below (table 8). The percentage figures refer to the amount of secondary dimonoside. It will be noticed that in families 49/38 and 57/38 less than 100 per cent. dimonoside is produced by the double heterozygote *XxZz*, and slightly less than 100 per cent. pentoseglycoside by the homozygous *dunnii*, *xxZZ*.

TABLE 8

Pedigree showing constitution of dunnii *and two garden forms for the complementary genes* X *and* Z *controlling the production (amount per cent.) of secondary dimonoside*

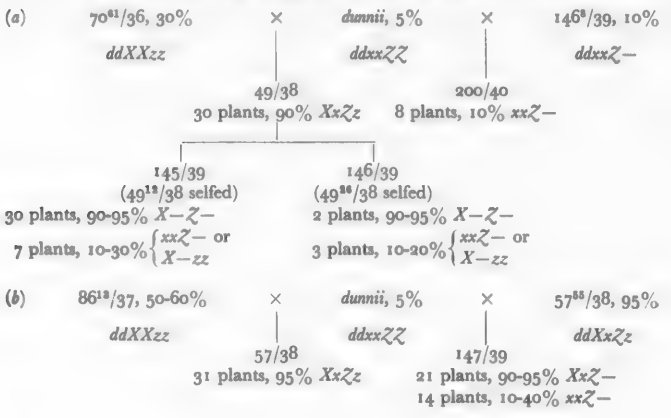
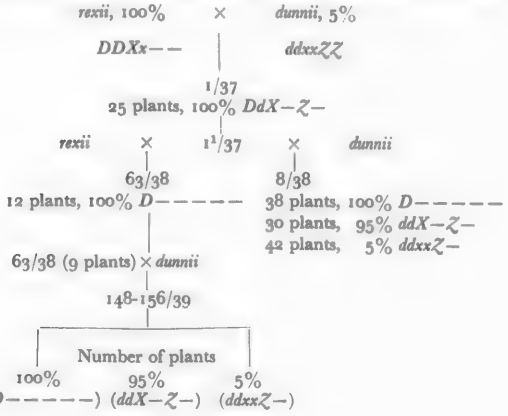


TABLE 9

Pedigree showing constitution of rexii *for* X

Amounts of primary (*D*) and secondary (*d*) dimonoside in per cent.



	100% (*D*− − − − −)	95% (*ddX*−*Z*−)	5% (*ddxxZ*−)
2 families (*DD*− − − −)	94	0	0
2 families (*DdxxZ*−)	44	0	35
3 families (*DdXXZ*−)	70	68	0
2 families (*DdXxZ*−)	28	30	7

Further evidence is supplied by derivatives from *rexii-dunnii* crosses (table 9). The occurrence of the 95 per cent. dimonoside class in family 8/38 shows that the plant of *rexii* used must carry an *X* gene. Similarly, the occurrence of the 5 per cent. class in families 148-156/39 shows that *rexii* must carry an *x* gene, i.e. *rexii* is *Xx*. *rexii L*

TABLE 10

Pedigree showing constitution of rexii *for* Z

$70^{61}/36$, 30% *ddXXzz* × *rexii*, 100% *DDXxZ*− × $83^{1}/36$, 10% *ddXXzz*

74/38
14 plants, 100% *Dd*− − − −

73/38
25 plants, 100% *Dd*− − − −

	252/39 ($74^{1}/38$ selfed)	253/39 ($74^{7}/38$ selfed)	250/39 ($73^{11}/38$ selfed)	251/39 ($73^{24}/38$ selfed)	Total
100%, *D*− − − − −	41	37	25	27	130
64%, *ddXxZ*−	1	4	11	3	16
30%, *ddX*−*zz* / *ddxxzz*	1	5	3	2	11

also was crossed to *dunnii* and a B_1 raised by backcrossing to *dunnii*. The numbers of 100, 95 and 5 per cent. dimonoside progeny were 45, 30 and 33 respectively (*cf.* table 9, family 8/38). Thus, *rexii L*, like *rexii*, may be presumed to be *Xx*. *rexii L* differs from *rexii* in a sufficient number of characters to suggest it should have the rank of a sub-species, if not species ; and its provenance is, apparently,

TABLE 11

Inheritance of Z

B_1 parents (*Od*) (see table 10)	Percentage dimonoside : class means			
	87·5	62·5	37·5	12·5
$70^{61}/36 \times 74^{7}/38$ (*XXzz*)	22	2	5	0
$70^{61}/36 \times 74^{1}/38$	21	3	4	1(15%)
$83^{1}\ 36 \times 73^{24}/38$ (*XXzz*)	5	0	1	0
$73^{11}/38 \times 83^{1}/36$	8	1	0	0

highly limited. Therefore the occurrence of *x* in both *rexii* and *rexii L* suggests that this mutant is by no means rare in the wild.

The remaining evidence for the constitution of *rexii* came from crosses with two true breeding, low dimonoside varieties (table 10). The figures for F_2 refer to *O*(*R*)*d* genotypes only, since it is not possible to identify satisfactorily low and high dimonosides in *ord* forms. The values 64 per cent. and 30 per cent. in families 250-253/39 represent the averages of high and low dimonoside production forms respectively. The occurrence of the 64 per cent. class confirms that *rexii* carries *Z*, as do also the B_1 families (table 11). Although in table 11 clear-cut

segregation of low and high dimonosides is not evident as in tables 8 and 9, it is, nevertheless, obvious that low dimonosides ($X-\mathcal{Z}-$) types occur. If *rexii* were $\mathcal{Z}\mathcal{Z}$ all F_1 individuals would be $\mathcal{Z}z$ and all should give a proportion of high dimonoside $X\mathcal{Z}$ forms. But if *rexii* were $\mathcal{Z}z$ some of the F_1 plants should breed true for low dimonoside. Actually, none did so but as only four were tested it cannot be concluded that *rexii* is $\mathcal{Z}\mathcal{Z}$.

The behaviour of the garden forms can now be examined in the light of the above scheme. From tables 8 and 10, we have seen that garden forms $70^{61}/36$, $83^{1}/36$ and $86^{13}/37$ can be assigned the constitution *XXzz*. In addition, sixteen other garden forms were crossed with *dunnii*. Twelve of the F_1's comprised high dimonoside individuals only and the garden parents can be designated $XX--$. Three of

TABLE 12

Inheritance of 3-pentoseglycoside (PG) and 3-monoside (MO) in garden forms and in garden forms × dunnii

Parents	No. of plants	Progeny
PG selfed	12	PG
PG × MO	66	PG
PG × PG (*dunnii*)	27	PG
MO × PG (*dunnii*)	14	PG
(MO × PG (*dunnii*)) × MO	8	PG (?)
MO × MO	37	MO (and some PG (?))

these twelve gave only low dimonosides when selfed and are, therefore, *XXzz*. The remaining four garden forms gave both high and low individuals in F_1 with *dunnii* and so are $Xx--$. Finally, one garden form that gave only high dimonosides when crossed to *dunnii* gave only high dimonosides when selfed: its constitution is, therefore, $XX\mathcal{Z}\mathcal{Z}$. No $xx\mathcal{Z}\mathcal{Z}$ garden forms have been identified from breeding. Thus all 19 of the garden forms crossed to *dunnii* carried *X*. This is not surprising since of 91 *dd* individuals examined chemically, only two did not carry secondary dimonoside, *i.e.* the great majority must be presumed to be $X-\mathcal{Z}-$.

(c) *Pentoseglycoside and monoside.* Ninety-two *dd* garden forms were tested for their glycosides. Ninety contained a proportion of 3 : 5-dimonoside, the accompanying glycoside being 3-pentoseglycoside in 57 plants and 3-monoside in 33 plants. Two plants contained nothing but 3-monoside. Therefore the genes controlling pentoseglycoside and monoside production are common in the garden forms and these sugars occur as alternatives.

Curtailment of these studies prevented our obtaining conclusive evidence on the inheritance of 3-pentoseglycoside and 3-monoside (table 12). The pentoseglycoside character appears to be dominant (or, more probably, epistatic) to the monoside. The simplest genetical explanation for these results would be that two pairs of genes are

concerned such that *P* (*Q*) produce pentoseglycoside and *pQ* monoside. But the inheritance of monoside is not clear, monoside crossed monoside apparently giving pentoseglycoside as if, for example, complementary genes were involved in the production of pentoseglycoside. One situation, however, is plain. Some *XXzz* garden forms contain 90-100 per cent. monoside, others 90-100 per cent. pentoseglycoside, therefore the possibility that *X* produces monoside and *Z* pentoseglycoside can be rejected and the control of monoside and pentoseglycoside must be ascribed to other genes. For ease of discussion, we shall use the symbol *P* to denote a gene, or genes, producing 3-pentoseglycoside and similarly *Q* for monoside.

TABLE 13

Independent inheritance of O *and* C

Generation	Genotype			
	OC	*Oc*	*oC*	*oc*
F_2	42	17	17	4
	Calc. *45*	*15*	*15*	*5*
B_1	5	6	9	7
B_1	9	12	6	7
B_1	9	10	4	6
B_1	9	10	6	7
	Total 32	38	25	27
	Calc. *30·5*	*30·5*	*30·5*	*30·5*

(v) *Linkage*

No special study has been made of linkage between the pigment-producing and modifying genes discussed in this paper. *F*(*A*), *O*, *R* and *D* were earlier shown to be independent, which is confirmed, and now independence is established for *V* and *F* (table 3). Segregation for *O* and *C* is given in table 13 and reveals the independent inheritance of these alleles. There is no linkage evidence regarding *X* and *Z*. As will be shown in a subsequent paper, *F* and *I* are components of a complex—or super—gene and exhibit complete linkage in appropriate crosses, but this has no relevance for the present discussion.

(vi) *Interaction of pigment genes*

During the course of the experiments described in this paper, 133 parental garden forms (20 blue, 33 mauve, 17 magenta, 34 rose, 3 pink and 26 salmon) were tested chemically for their flower pigments. Until these tests were made, there was no means of knowing the proportion of dimonoside, pentoseglycoside or monoside in these plants or the degree of co-pigmentation. Thus, it would be legitimate to regard them as a fairly unbiased population from which samples

could be taken for one purpose or another, *e.g.* to ascertain the degree of co-pigmentation or the proportions of the glycosides.

Plants were scored for the amounts of primary or secondary dimonoside, pentoseglycoside, monoside and cyanidin, also co-pigment and chlorogenic acid. The amounts of the glycosides were estimated in per cent. at intervals of 5 per cent. The other characters were classified into 4 arbitrary, but distinct, classes denoted by the values 100, 75, 50 and 25 per cent. with a fifth class, 0 per cent. The figure of 100 per cent. does not necessarily indicate the maximum possible value but only that class which approaches, and includes, the maximum. Since the amount of 3 : 5-dimonoside found in *dd* forms is less than 50 per cent. in 70 per cent. of cases, the percentage of individuals having 25 per cent. or more dimonoside was chosen as a convenient classification.

The actual method of comparison was to estimate penetrance and expressivity of the " dominant " genes concerned, in relation to the three anthocyanidin classes, *OR*, *oR* and *or* representing the delphinidin, cyanidin and pelargonidin derivatives respectively. It could be argued that the terms penetrance and expressivity do not strictly apply to some of our data, since there is no conclusive proof that single genes control methylation and production of chlorogenic acid ; and two complementary genes are responsible for secondary dimonoside production. The terms are convenient, however, and in any case the concept of a single gene being responsible for a given phenomenon is dependent on the degree to which genetic analysis itself has " penetrated ". Further, all, or the great majority, of the plants investigated carried the gene(s) producing a particular character, consequently, unless pigment suppression occurred through gene interaction, that character should have been manifest. The genetic constitution of all the plants investigated for primary dimonoside content was actually determined by breeding ; all but two of the total number of plants scored for secondary dimonoside did, in fact, contain dimonoside and therefore carried both *X* and *Z* ; all plants but two were methylated and therefore can be postulated to carry *M* ; *cc* individuals were a small minority ; and all the plants scored for pentoseglycoside or monoside did in fact contain these glycosides and the population must therefore have carried the gene(s) governing pentoseglycoside and monoside production. In short, quantitative variation in the characters studied would result, in the main, from gene interaction and not gene segregation.

The analyses covered plants bred from 1934 to 1939 inclusive. To avoid drawing erroneous conclusions the data were examined for any shift in the frequency of pigment types that might have occurred owing to unconscious genetic selection over this period. No trend of consequence was found except for co-pigmentation in the rose class (*oRd*) subsequent to 1937, consequently, only the 1934-36 data were used throughout the *oR* anthocyanidin class.

Referring to the genetical constitution of the *OR* class, 26 plants were known to be homozygous dominants *RR*, 3 were heterozygotes and 4 were either homozygotes or heterozygotes. Thus any genetical variation in the *OR* class would be mainly with respect to *O*, at least 70 per cent. of the plants being heterozygous for this gene. In the *oR* class, at least 60 per cent. of the plants were *RR*. Since mixtures of malvidin and peonidin, or malvidin and pelargonidin or peonidin and pelargonidin have not been found in the garden forms, *O* and *R* may be assumed to be completely dominant or nearly so.

The results of the survey are given in table 14 which, while it must be taken to indicate trends only, brings to light interesting aspects of gene interaction in pigment production. The glycoside data refer to the occurrence or amount of mixed glycosides and each row involves a comparison between two types of sugar. Thus with primary (*Dd*) and secondary dimonoside, the comparison is between dimonoside on the one hand and pentoseglycoside or monoside on the other hand ; with pentoseglycoside the comparison is between pentoseglycoside and monoside. Similarly with cyanidin the comparison is between this unmethylated pigment and a methylated one, *i.e.* between mixed anthocyanins. No comparison is possible with either of the anthoxanthins, the data indicating the occurrence or amount of one pigment only.

Complete penetrance was observed in 7 *DD* and 24 *Dd* plants producing primary and secondary dimonoside respectively ; also for chlorogenic acid. Since segregation for chlorogenic acid has never been observed in *Streptocarpus* it is highly probable that for this character also, all the plants tested were homozygous dominants. In two other instances, *viz.* pentoseglycoside and co-pigment, penetrance varies with the nature of the anthocyanidin, being least in the *or* class and greatest in the *OR*. Thus in the case of pentoseglycoside, the percentage of the population carrying this sugar as opposed to monoside increases $or < OR$; although no similar comparison (mixed pigments) arises with co-pigment, there is a similar increase in penetrance from $or < OR$. In short, *D*, *XZ*, *P* and *C* can be said to interact quantitatively, each in the same way, with *O* and *R*. On visual assessment we had described *D* as being completely dominant, i.e. *Dd* plants are conspicuously bluer than *dd* and it is not possible to distinguish heterozygotes from dominant homozygotes. On chemical assessment, dominance is not always complete in *Dd* heterozygotes, the proportion of plants showing complete dominance being 90, 75 and 50 per cent. respectively in the *OR*, *oR* and *or* classes. A similar situation has been reported in *Antirrhinum* (Jorgensen and Geissman, 1955).

Summing the data for *D* (in *Dd* plants), *XZ*, *P* and *C* it is found that the effect of *oR* on penetrance, compared with that of *or*, is approximately the same as that of *OR* compared with *oR*. These incremental effects must be ascribed, in the main, to the presence

or absence of *O* and of *R*, since comparison of homozygotes with heterozygotes reveals no correlation with penetrance, *i.e.* dominance of *O* and *R* is complete or nearly so.

Considered as a whole (but excluding cyanidin for the moment), the data in table 14 suggest that penetrance is (1) complete when the gene concerned is in the homozygous dominant phase, (2) incomplete in the heterozygous phase, (3) dependent in degree on the genetical background with respect to *O* and *R*; and (4) the incremental effects of *R* and *O* on penetrance are approximately equal and supplementary.

TABLE 14

Gene interaction in pigment production

Pigment character	Genes	No. of plants	Percentage penetrance			Mean expressivity		
			OR	*oR*	*or*	*OR*	*oR*	*or*
Dimonoside								
(i) primary . .	D | *DD*	7	100	100	100	100	100	100
	| *Dd*	24	100	100	100	100	97	93
(ii) secondary . .	*X Z*	91	88	61	42	41	50	43
Pentoseglycoside . .	*P*	57	88	58	35	59	57	69
Anthoxanthin								
(i) chlorogenic acid .	? | *D*	37	100	100	...	73	74	93†
	| *d*	90	100	100	100	66	66	80
(ii) co-pigment . .	*C* | *D*	37	95	70	...	82	58	68†
	| *d*	90	97	82	64	72	44	41
Cyanidin . . .	*M* | *D*	91	65	100	...	18	37	20†
	| *d*	40	79	100	62	15	30	15

* Percentage of plants with more than 25 per cent. dimonoside.
† Only three plants.

Turning to expressivity in general the degree of expressivity is lower than the degree of penetrance, complete expression is found only in *DD* plants, and no consistent gradation is to be seen between anthocyanidin classes. However, while the glycosides exhibit no great differences between anthocyanidin classes, the anthoxanthins, especially co-pigment, do and, further, expressivity seems to vary with the genetic constitution for *D* and *d*, i.e. there is correlation in the production of co-pigment and diglycoside and *C* interacts with *D* and *XZ*. The fact that penetrance grades *OR*>*or*, but expressivity does not, probably means that the differences in degree of penetrance stem from differences in the threshold for production of a precursor necessary for the synthesis of anthocyanin.

The results with cyanidin require special consideration. Although all but two of the garden forms contained methylated anthocyanins and gave methylated progeny, *i.e.* they bred true for *M*, the unmethylated pigment, cyanidin, often accompanies, usually in small

amount, the methylated anthocyanins malvidin and peonidin, also pelargonidin. For example, in *dd* plants the percentage frequency of the classes 0, 25, 50, 75 and 100 per cent. pigment (p. 316) is respectively 28, 62, 7·0, 2·7 and 0·9. In *dd* plants, penetrance is complete in the *oR* class but not in *OR* and *or*; and similarly expressivity is relatively high in the same class, i.e. *oR* plants produce on average twice as much cyanidin as *OR* or *or*. Results for expressivity in the presence of *D* appear closely to resemble those with *dd* forms.

Further light is thrown on gene interaction involving cyanidin production by considering its association with the glycosides. In table 15, groups (*b*) and (*d*) might be termed " expectation " on the basis of the garden forms being homozygous for *M*. Groups (*e*) and (*f*) are manifest examples of interaction since cyanidin is found associated with one glycoside type but not the other. The majority

TABLE 15

Genotype	No. of plants	Cyanidin present with :	
		Dimonoside	Pentoseglycoside or monoside
D—	33	(*a*) +	
	7	(*b*) — (*OR*)	
dd	53	(*c*) +	+
	17	(*d*) —	—
	3	(*e*) + (*or*)	—
	18	(*f*) —	+ (14 *O*—, 4 *or*)

of plants fall into groups (*a*) and (*c*) and in these interaction is not, in the first place, between the genes controlling cyanidin and glycoside, but between those controlling cyanidin production and anthocyanidin type, as clearly seen from table 14. Nevertheless, analysis of group (*c*) shows that whereas in *or* and *OR* types the ratio of cyanidin accompanying dimonoside and non-dimonoside fractions is approximately 1 : 1, in the *oR* group it is 1·0 : 1·6, *i.e.* more cyanidin accompanies the non-dimonoside than the dimonoside pigments or, conversely, methylation (or methoxylation) is correlated with dimonoside production and *M* interacts with *XZ*.

Correlation between methylation and glycoside type was also observed in four comparable sibs, two *ORd* and two *oRd*, (*rexii* × *dunnii*) × *dunnii* containing mixtures of methylated and unmethylated pigments, table 16. Methylation in both peonidin and malvidin derivatives was more complete in the dimonoside fraction than in the pentoseglycoside, i.e. *M* interacts with *D*.

Correlation between methylation and glycoside type can also be seen by taking, separately, the pentoseglycoside and monoside fractions

and noting the numbers of completely methylated individuals (table 17). The figures for 37 *D* forms are also included in the table which shows that *M* interacts with both *D* and with *P*.

Finally, correlation between methylation and anthocyanidin type was demonstrated by counting the number of plants containing more

TABLE 16

$8^{16}/38$	45% peonidin 3 : 5-dimonoside. 55% cyanidin and a little peonidin 3-PGs.
$8^{34}/38$	45% peonidin and a little cyanidin 3 : 5-dimonosides. 55% cyanidin and a little peonidin 3-PGs.
$8^{25}/38$	60% malvidin 3 : 5-dimonoside. 40% delphinidin 3-PG.
$8^{136}/38$	55% malvidin 3 : 5-dimonoside. 45% delphinidin and a little malvidin 3-PGs.

methylated pigment in the dimonoside fraction than in the non-dimonoside, the percentage of such individuals in the *ORd*, *oRd* and *ord* classes being 72, 62 and 25 respectively, i.e. *M* interacts with *O* and *R* and with *D*.

So much for the anthocyanins. Interaction is also manifest with the anthoxanthins as can be seen by comparing the amounts of chlorogenic acid and co-pigment produced in *FF* (deep cyanic) and *Ff*

TABLE 17

Correlation of methylation and glycosidation

Glycoside type	Percentage individuals completely methylated	
	O(R)d	*oRd*
Dimonoside *D* . . .	64	2
Pentoseglycoside (*d*) . .	24	0
Monoside (*d*) . . .	0	0

(paler cyanic) classes. The results are given in table 18, the lower half of which also includes the mean amounts for *RR* and *Rr* individuals, distinguishing between *FF* and *Ff* genotypes. The action of *F* is known to be cumulative (p. 305) hence the table shows clearly that with increase in the amount of anthocyanin in *FF* compared with *Ff* plants there is decrease of both anthoxanthins, i.e. *C* and the genes controlling anthoxanthin production both interact with *F*.

It is probably significant that whereas there is no marked decrease in the amount of chlorogenic acid with increase in the number of

recessive genes (column 1), there is a striking decrease in the amount of co-pigment. This suggests that the gene(s) controlling production of chlorogenic acid operates at an earlier stage of synthesis than does the gene controlling production of co-pigment.

The data referring to *R* are of note. They show that interaction occurs between *R*, *F*, *C* and the gene(s) controlling the production of chlorogenic acid. Less chlorogenic acid is produced in *FF* flowers than *Ff* and, superimposed on this, less in *RR* flowers than *Rr*. This multiple competition is even more pronounced in the case of co-pigment. Assuming that decrease in the amount of anthoxanthin is

TABLE 18

Interaction of anthocyanins and anthoxanthins

Genotypic class	No. of plants	Chlorogenic acid mean amount per cent.			Co-pigment mean amount per cent.		
		FF	*Ff*	*FF* and *Ff*	*FF*	*Ff*	*FF* and *Ff*
O(R)D	8	81	88	85	100	94	97
O(R)d	31	62	73	67	63	82	72
oRD	14	63	88	73	28	50	38
oRd	28	65	63	65	33	47	38
ord	21	75	86	81	23	32	28

		FF		*Ff*		*FF*		*Ff*	
		RR	*Rr*	*RR*	*Rr*	*RR*	*Rr*	*RR*	*Rr*
oD and *od*	40	62	68	71	84	28	41	40	65

the result of increase in the amount of anthocyanidin ; and that both anthoxanthin and anthocyanidin are synthesised from a common substrate or precursor, then these results show that *R* increases the amount of anthocyanidin produced by *F*. But the apparent effect of *R* is to add a hydroxyl at position 3'-. Therefore the synthetic process that leads to increase in hydroxylation is closely associated with a process that leads to increase in the amount of precursor. Moreover since more anthocyanidin is produced in *RR* than *Rr* flowers ; and since *R* is completely dominant or nearly so, then in single dose it controls a reaction at an upper limit of its curve of effectiveness (Muller, 1932), especially in *FF* plants, both in respect of the amount of anthocyanidin and its kind, peonidin (or cyanidin). In other words, from the adaptive point of view, *R* has a high " factor of safety " (Haldane, 1939).

Owing to lack of the requisite genotypes no similar study could be made with *O* (malvidin) but since this gene is completely dominant, and having regard to the results given in table 14, it is probable that *O*, like *R*, is a hypermorph.

Summarising the evidence on gene interaction in pigment production in the garden *Streptocarpus*, it appears that :—

(1) Penetrance of *D*, *XZ*, *P* and *C* increases with increase in the number of loci controlling hydroxylation at which dominant genes are present, thus : *or*<*oR*<*OR* the incremental effects of *R* and *O* being approximately equal.

(2) Expressivity of *D*, *XZ*, *P* and *C* reveals no such proportionality but expressivity of *C* is seemingly higher in *D* than *d* plants.

(3) Penetrance and expressivity of cyanidin is greater in *oR* than *OR* and *or* genotypes.

(4) Interaction occurs between *O* (also *R*) and *D*, *XZ*, *P*, *M* and *C* ; between *M* and *D*, *XZ* and *P* ; between *C* and *F*, *D* and *XZ* ; and between the gene(s) for chlorogenic acid and *F* and *R*.

(5) The main effects of these interactions on the chemical phenotype are :—

(i) increase in the number of hydroxyl groups in the B-ring is correlated with increase in methylation, also with increase in the number of sugars at positions 3 and 5 (monoside→pentoseglycoside→dimonoside).

(ii) increase in the aggregate number of the dominant genes *O*, *R* and *D* is associated with increase in apigenin production.

(iii) increase in anthocyanin production is correlated with decrease in anthoxanthin production.

4. GENETICS OF SPECIES

(1) *Flower pigments*

(a) *Species*. The data for the chemistry and genetics of flower colour in the garden forms have been given in the previous pages. We have now to see whether these facts throw any light on pigmentation and inheritance in the species and species hybrids.

More than 45 forms of *Eustreptocarpus* have been accorded specific rank by taxonomists. Of these, the authors have grown 27, *viz.* 21 with bluish flowers, 1 with brick-red (*dunnii*), 1 with cream (*vandeleuri*), and 4 with white flowers (*parviflorus*, *pusillus*, *umtaliensis*, B. L. Burtt, and *wilmsii*, Engler). The anthocyanins identified are listed in table 19.

Thirteen of the sixteen species tested chemically are pigmented by malvidin 3 : 5-dimonoside, one (*meyeri*) by delphinidin 3 : 5-dimonoside, and one (*dunnii*) by cyanidin 3-pentoseglycoside. The anthocyanins are the same as those found in the garden forms, and segregates derived from crosses between the species and the garden forms give the expected flower colours and pigments. We may assume therefore that the major genes controlling flower pigmentation in the species are homologous with those in the garden forms, *i.e.* 15 of the 16 species carry *O*, 15 *D*, 14 *M* and 1 *od*.

The species listed in table 19 are all *OD* genotypes except *dunnii*

which is *od*. *parviflorus*, *gracilis*, *comptonii* and *galpinii* also are *OD* and *vandeleuri oD*, a grand total of 19 *ODM*, 1 *ODm*, 1 *oDM* and 1 *odm*. In addition, one plant of *parviflorus* proved to be *Od*.

TABLE 19

Anthocyanin class	Species	Anthocyanin pigment
1	*cyaneus*, S. Moore, *daviesii*, C. B. Clarke, *galpinii*, Hook fil., *gardeni*, Hook, *grandis*, N. E. Brown, *insignis*, B. L. Burtt, *michelmorei*, B. L. Burtt, *polackii*, B. L. Burtt, *wendlandii*, Sprenger	Malvidin 3 : 5-dimonoside
2	*rexii*, Hook	Malvidin 3 : 5-dimonoside + trace cyanidin dimonoside
3	*gracilis*, B. L. Burtt, *haygarthii*, N. D. Brown, *johannis*, L. L. Britten, *polyanthus*, Hook	Malvidin 3 : 5-dimonoside + some petunidin and/or delphinidin
4	*meyeri*, B. L. Burtt	Delphinidin 3 : 5-dimonoside (+possibly some petunidin)+cyanidin 3 : 5-dimonoside
5	*dunnii*, Mast.	Cyanidin 3-pentoseglycoside + 5-10% cyanidin 3-bioside

The fourth of the main pairs of alleles governing anthocyanin pigmentation is *Rr*. Since *O* is epistatic to *R*, the distribution of the latter gene among the species cannot be directly determined. Crosses were therefore made between 18 species and 4 wild varieties on the

TABLE 20

Distribution of R, r *in* Streptocarpus

Species	*R*—	*rr*
cyanic	*gardeni*, *meyeri*, *johannis*, *polyanthus*, *haygarthii*, *grandis*, *wendlandii*, *dunnii*, *michelmorei*, *eylesii*, B. L. Burtt	*rexii*, *rexii L*, *insignis*, *cyaneus*, *polackii*, *montigena*
acyanic	*parviflorus*, Hook *, *vandeleuri*, E. G. Bak and S. Moore	*meyerii* var. *rexii S*, *rexii B*, *rexii FB*

* *parviflorus*, Hook is incorrectly given as *parviflorus*, E. Meyer in Lawrence *et al.* 1939.

one hand, and *ord* garden forms on the other. *R* or *r* forms were then extracted by backcrossing and identified chemically (table 20). Twelve species carry *R* and six *r*. Five of these six are members of what, with respect to the general morphology of flowers and foliage, we shall call the *rexii* group. This group is identified by morpho-chemical characteristics and, as will be shown in a later paper, by

its geographical distribution. Three white-flowered varieties of *rexii* from the wild also are *r*. Although *R* is hypostatic to *O*, it appears to have an adaptive advantage since it is carried by the majority of the species examined.

It is of interest that in *gracilis*, *haygarthii*, *johannis*, and *polyanthus*, species having distinct cornucopea-shaped flowers, methylation is not complete and the anthocyanins are therefore mixtures of malvidin, petunidin and/or delphinidin dimonosides. Here again is a natural group identified by morpho-chemical and geographical characteristics.

(b) *Species hybrids*. Thirty-three hybrids involving twelve species have been examined for their anthocyanin pigments. The results are summarised as follows :—

(1) In 9 cases where class 1 parents (completely methylated) were crossed together, all the hybrids were completely methylated.

(2) In 13 cases where class 1 parents were crossed with class 2, 3 or 4 parents (incompletely methylated), 10 hybrids were completely and 3 incompletely methylated.

(3) In 9 cases where class 1 parents (complete oxidation) were crossed with class 2 or 4 parents (incomplete oxidation), 7 of the hybrids showed complete and 2 incomplete oxidation.

(4) In 6 cases where class 1 parents were crossed with class 5 (incomplete oxidation and methylation), 5 hybrids showed incomplete and 1 complete oxidation and methylation.

(5) *dunnii* (class 5) ×*michelmorei* (class 1) gave hybrids containing diglycoside and pentoseglycoside.

Referring to the species pigmented mainly by malvidin, it is clear that those anthocyanin characters occurring most frequently in the genus, namely complete oxidation and methylation, tend to be completely dominant in inheritance. This is evident in the hybrids with *dunnii* (malvidin ×cyanidin) where, though there is a moderate amount of cyanidin, the main pigment is completely oxidised and methylated.

(II) *Stem and leaf pigments*

Six wild forms, viz. *rexii*, *gardeni*, *montigena*, *johannis*, *grandis* and *grandis albus*, also two artificial hybrids derived from *grandis* and *dunnii* respectively, have been found by our colleague, Dr J. B. Harborne, to contain one main pigment in their peduncles. *rexii*, *montigena* and the *dunnii* hybrid carried this same pigment in their leaf tips. From chromatographic and spectral studies, it was established that the pigment was an unacylated cyanidin glycoside. It was, however, different from any of the known cyanidin glycosides, and, on acid hydrolysis, gave the sugars, glucose and xylose. On partial acid hydrolysis the pigment gave rise to cyanidin 3-glucoside. It appears from these results that the pigment is cyanidin 3-xyloglucoside.

The authors had previously used Robinson's methods to identify

the stem pigment in other species. In *rexii B*, *gracilis*, *comptonii* and the acyanic species *kentaniensis*, Britten and Storey, and *umtaliensis*, the main pigment was a cyanidin glycoside. Twelve B_1 plants from (*rexii* × *dunnii*) × *dunnii* also contained the cyanidin glycoside. In the hybrid, *johannis* × *wendlandii* a mixture of cyanidin monoside and the glycoside was found in both stems and leaves, and this mixture was carried by the deeply pigmented leaves of *wendlandii*. Thus, in every species examined the stem pigment is cyanidin, presumably present as the previously mentioned 3-xyloglucoside.

The fact that unmethylated anthocyanidin is found in the stems of a number of species whose flowers carry methylated pigment, means that *M* acts only in the flowers. A somewhat similar situation is seen in *wendlandii* where *D*, producing 3 : 5-dimonoside, is completely dominant in the flowers but not in the leaves where monoside appears to predominate. Again, in *D* plants, 3 : 5-dimonoside is produced in the flowers but the cyanidin glycoside is produced in the flower stems. Thus, *D* also acts only in the flowers.

(III) *Pigment Inheritance*

In section (i) evidence was presented respecting the constitution of various species for the genes *O*, *R* and *D* determining specific anthocyanidin pigmentation. In this section breeding results will be given for *F*.

parviflorus and 1/33, both *VVffii*, were crossed to a natural white-flowered variety of *meyeri* here styled *meyeri albus*, and to *pusillus*, *wilmsii* and *grandis albus*, 11 combinations in all. None of these gave cyanic progeny, therefore all are *ff*. *meyeri albus*, *wilmsii* and *grandis albus* have coloured flower stems and are presumably *VVff*. *pusillus* has green stems and may be *vvff*. Three acyanic varieties of *rexii* were tested : *rexii S* and *rexii B* are *VVff* and *rexii FB vvFF*. Since *rexii* is *VVFF*, all three of the possible homozygous dominant combinations are shown to occur in the wild.

The inheritance of *F* and especially *f*, was also studied in five series of crosses. Table 21 compares, in summary, the breeding behaviour of *rexii* and its varieties in intra- and inter-specific crosses and in reciprocal crosses with species and garden forms.

Inter-specific crosses give normal ratios for mono-factorial inheritance (series 1*a*, 3 and 4*a*) but in intra-specific crosses (series 5*a* and *b*) there is a great deficiency of recessives (F_2, 26 : 1 ; B_1, 4 : 1). An additional comparison can be made between what we may call inter- and intra-group crosses. Thus most of the cyanic species used in series 1*b* and 3 belong to the *rexii* group and these, when crossed to acyanic garden forms (equivalent to inter-group crossing) give normal 3 : 1 ratios (series 3), unlike the intra-group crosses with *rexii S* (series 1*b*) where the ratio is 3·6 : 1 and significantly aberrant. A similar situation is found between the inter- and intra-group F_2's in

series 4*a* where the ratio is 3 : 1 and 4*b* where it is 6 : 1 (though the B_1 in 4*c* is normal) ; and also between series 1*a*, ratio 3·0 : 1 and 1*b*, ratio 3·6 : 1. Finally, a clear-cut difference is seen between acyanic

TABLE 21

Inheritance of F, f *in species crosses*

Parents	No. of families	F_2 (3 : 1)	χ^2		D.F.	*P*
			Deviation from expectation	Hetero-geneity		
1st series						
(*a*) 5 cyanic species (*rexii L, polackii, cyaneus, johannis, grandis*) × *ff* species other than *rexii*	7	236 : 74	0·211	...	1	0·90-0·50
				3·905	6	0·90-0·50
(*b*) 6 cyanic species (*rexii, rexii L, insignis, polackii, cyaneus, gardeni*) × *rexii S* (*ff*)	12	1306 : 364	9·141	...	1	0·01-0·001
				50·128	11	v. small
				52·788	12	*v. small*
2nd series						
(*a*) acyanic garden forms × *rexii*	6	199 : 61	0·328	...	1	0·90-0·50
				8·770	5	0·50-0·10
(*b*) reciprocals	9	221 : 48	7·347	...	1	0·01-0·001
				14·522	8	0·10-0·05
				18·562	8	0·02-0·01
3rd series						
6 cyanic species (*rexii L, insignis, polackii, gardeni, polyanthus, dunnii* × *Ff* garden forms	13	229 : 82	0·310	...	1	0·90-0·50
				19·520	12	0·10-0·05
4th series						
(*a*) [2 species (*polackii, johannis*) × an *ff* derivative of *rexii L*] × *rexii S* (*Ff*)	2	100 : 34	0·010	...	1	0·95-0·90
				0·081	1	0·90-0·50
(*b*) *insignis* × do.	4	169 : 28	...	*3·285*	3	0·50-0·10
(*c*) 2 species (*insignis, polackii*) × *rexii S* (*ff*)	2	45 : 44*	...	*4·102*	1	0·05-0·02
5th series						
(*a*) *rexii S* (*ff*) × cyanic sib	6	337 : 12	...	*4·355*	5	*0·50*
(*b*) F_2's from (*a*) back-crossed to *rexii S* (*ff*)	3	143 : 35*	...	*2·669*	2	*0·50-0·10*

* = B_1.
italics = calculated on the actual ratios observed, *i.e.* not on 3 : 1 or 1 : 1.

garden forms crossed to *rexii* (2*a*) and the reciprocals (2*b*), the respective F_2 ratios being 3·3 : 1 and 4·6 : 1.

If, in this series of crosses, cyanics are presumed to be *VFi* and acyanics *Vfi*, then the segregation of *f* in F_2 is (1) normal when cyanic species other than *rexii* are crossed with garden forms or with acyanic species other than *rexii* varieties, (2) abnormal when *rexii* is used as

the female, but not male, parent, and (3) greatly abnormal in crosses between cyanic and acyanic varieties and progeny of *rexii*.

The reciprocal difference seen in the F_2's in 2 (*a*) and (*b*), table 21, could be a cytoplasmic effect, in which *ff* zygotes are fully viable with garden-form cytoplasm but often inviable with that of *rexii*. Alternatively, certation would fit the results if we suppose *f* pollen-tubes grow at the normal rate in the styles of plants having garden-form cytoplasm but much slower in styles of plants with cytoplasm derived from *rexii*. An analogous case has been reported in crosses between *Œnothera pallida* and *O. trichocalyx* (Crowe, 1955) where it was found that it was the cytoplasm in the pollen which controlled the growth rate of the various pollen genotypes and the proportions of the F_2 segregates. Certation is common in the *rexii* group of species, as will be shown in a later paper, therefore it is more likely that the reciprocal differences discussed above are an expression of certation rather than of differential zygotic lethality.

In the fifth series, where the crosses are all within the one species, *rexii*, certation would be expected to exhibit its greatest effect, even in the absence of cytoplasmic influences. Whatever the particular mechanism that leads to the deficiency of *f* genotypes (note, the percentage deficiency is of the same order in both F_2 and B_1), it is clear that *rexii* carries a factor, or factors, which strongly favour the survival of cyanic (*F*) genotypes, a feature which is probably shared by *rexii* varieties and perhaps also by closely related species in the *rexii* group. Observations on *Streptocarpus* in the wild support this view. " The coloured flowers bloom in profusion in our forest patches, and I think one could expect to find (only) one or two white-flowered plants in any 500 acres where the others occur." (Storey, 1953).

A general conclusion from these studies on flower colour and its inheritance in the species is that a very high percentage of cyanic phenotypes is maintained in the wild population by adjustment of their nuclear and cytoplasmic balance.

5. DISCUSSION

(i) *Flower pigments*

Nine pairs of alleles controlling flower colour in *Streptocarpus* have been identified, each with a distinct phenotype and each exhibiting Mendelian inheritance. In these respects it is probable that, had selection of our material continued a little longer, a tenth factor, *M*, could have been included with the other nine. An eleventh factor, *P*, is suspected. The phenotypic effects of all these genes is summarised in table 22. Alleles similar in effect to *F*, *I*, *C* and *R* have been reported for various species (Scott-Moncrieff, 1936, 1939; Geissmann *et al.*, 1954).

The circumstances relating to the independent inheritance of *O* and *R* and epistasy of *O* (*OR* and *Or* giving delphinidin, *oR* cyanidin

and *or* pelargonidin derivatives) have been reported for only one other species, *Lathyrus odoratus* (Beale *et al.*, 1939). In *Lathyrus* interspecific hybridisation has not, apparently, been involved, the wild type being a delphinidin derivative, whereas in *Streptocarpus* the alleles *OO*, *rr* are derived from *rexii* and the *oo*, *RR* from *dunnii*. Although no cyanidin types occur in *Primula sinensis* it too could be considered along with *Streptocarpus* since *K* individuals contain malvidin and *k* pelargonidin, i.e. *K* is homologous to *O* in *Streptocarpus* in giving,

TABLE 22

Gene syndrome controlling pigment production and modification in flowers of Streptocarpus

Gene	General function	Phenotypic effect
V	Pigment production	(i) produces cyanidin 3-xyloglucoside in flower stems. (ii) produces delphinidin or cyanidin or pelargonidin in throat lines (*L*) and unidentified anthocyanidin in capitate pistillar hairs (*B*), *i.e.* production in limited areas.
F	,,	in presence of *V*, produces delphinidin, cyanidin or pelargonidin in whole corolla, *i.e.* production in unlimited area.
I	,,	increases amount of anthocyanidin in flowers.
C	,,	produces co-pigment (probably apigenin) in flowers.
R	Pigment modification	gives hydroxyl groups in the 3′ and 4′ positions on the side ring instead of only in the 4′ position (*r*).
O	,,	gives hydroxyl groups in the 3′, 4′ and 5′ positions instead of only in the 4′ position (*or*).
D	,,	gives large amount of 3 : 5-dimonoside in place of mixture of 3 : 5-dimonoside and 3-pentoseglycoside, or monoside (*d*).
X, *Z*	,,	complementary for production of limited amount of 3 : 5-dimonoside in place of 3-pentoseglycoside or 3-monoside (*xZ*, *Xz* and ?*xz*).
M?	,,	methylates hydroxyl groups.
P?	,,	controls 3-pentoseglycoside production.
Q?	,,	controls 3-monoside production.

apparently in one step, the 3′, 4′, 5′-trihydroxy structure in place of the 4′-monohydroxy one.

Turning now to the inheritance of glycoside, we have established that incomplete dominance of 3 : 5-dimonoside is sometimes found in *Dd* heterozygotes, and always in *ddX—Z—* individuals, the minor component of the mixed glycosides being either 3-pentoseglycoside or 3-monoside according to the genotypic constitution of the plant for the genes controlling these types. Although these genes have not been identified, it seems that the one (or ones) controlling pentoseglycoside production (*P*) will be found to be epistatic to that controlling monoside (*Q*). The glycosides would then fall into an ascending epistatic series: monoside (*Q*) pentoseglycoside (*P*), secondary dimonoside (*X*, *Z*) primary dimonoside (*D*), in a manner similar to the anthocyanidin series, pelargonidin (*or*), cyanidin (*oR*) and delphinidin (*OR*).

(ii) *Stem pigments*

At first sight, *V* produces only cyanidin in the stems but any one of the three anthocyanidin derivatives in the flower lines, and possibly in the pistillar hairs, of *Vf* flowers. However, in widely different species, species hybrids and garden forms, the stem anthocyanin has been identified as cyanidin 3-xyloglucoside, whether the flowers are cyanic *VF*, or acyanic *Vf*, and regardless of the presence or absence of the genes controlling hydroxylation, methylation and glycosidation of the anthocyanidin in the corolla (as distinct from the throat lines). Hence, *Oo*, *Rr*, *Mm*, *dd* and (possibly) *D*, modify the anthocyanidin in the flowers but not in the stems. And the basic effect of *V* must be the control of a precursor necessary for the production of anthocyanidin in flowers, stems and leaves. The action of *F*, then, is to extend the area of general cyanic pigmentation from the lines and hairs to the whole of the flower. In view of the diverse kinds of *Streptocarpus* to which the above facts apply, it is highly probable that they are universal for the genus.

Dr Harborne's investigations show that the glycoside found in the flower stems does not occur in the petals or lines of the blue, purple and pink flowers tested, *i.e.* in flowers pigmented by malvidin or pelargonidin. Moreover, the main glycoside components are 3 : 5-dimonosides.

If it turns out that the stem glycosides never occur in the flowers, and these contain 3 : 5-dimonoside, 3-pentoseglycoside and 3-monoside as the main glycosides, then, except for the cyanidin which occurs as a minor, and significant, component of the floral pigments, pigment differentiation between floral and vegetative organs will be complete. In essence this scheme refers to a macro-pattern of cyanic pigmentation, in which floral pigmentation, like floral morphology and physiology, is seen to be a highly selected and adapted function of reproduction.

(iii) *Gene interaction*

A major finding of our investigation on *Streptocarpus* is the interaction of nearly all, perhaps all, of the major genes concerned in the production of flower pigment. The phenotypic effects embrace pigment concentration, and hydroxylation, methylation and glycosidation of the pigment molecule. Together these phenomena constitute a pigment syndrome.

When two anthocyanins occur together in the same flower, a general characteristic of the syndrome is that that pigment which has the greater number of hydroxyls in the side ring, tends to be methylated and to have the greater number of sugars. In chemical terms, the genes controlling methylation and glycosidation are more active, or efficient, in synthesis in the *O* " background " than in the *R*, and in the *oR* background than the *or*. In genetical terms, interaction tends to produce the phenotype associated with epistasy and

dominance of the pigment genes, *i.e.* the wild phenotype, malvidin 3 : 5-dimonoside. Since the anthocyanidins with the greatest number of methoxyls in the side ring and the greatest number of sugars are the most stable chemically, gene interaction within the syndrome promotes phenotypic stability or homeostasis.

Had we been able to identify fully the *P* and *M* factors, then the syndrome might well be shown to comprise eleven independently inherited major genes, each of which could be described as a modifier (genetical) affecting anthocyanidin amount and/or kind.

The possibility of such a situation in flower-pigment production was first revealed by studies on *Dahlia* (Lawrence and Scott-Moncrieff, 1935), where four independently inherited genes controlling the production of anthocyanidin in limited amount (*A*), of anthocyanidin in quantity (*B*), of butein (*Y*) and of apigenin (*I*) were shown each to take part in the production of a common precursor for which they also competed, production and competition being predictable on a semi-quantitative basis according to the coefficients assigned to the genes. More recently Jorgensen and Geissman (1955) have described the results of a critical investigation of the interaction of three single genes governing the concentration, measured spectrophotometrically, of anthocyanidin and aurone in *Antirrhinum majus*. Three of Geissman's findings are particularly relevant to our work with *Streptocarpus*, viz. (1) increased numbers of recessive factors resulted in decreased anthocyanidin production, (2) high anthocyanidin concentration is related to low aurone concentration, and vice versa, and (3) the chemical consequence of heterozygosity is an alteration in the magnitude of the effect produced by the dominant alleles.

Our results with cyanidin are of special interest. It often occurs as a minor component in the company of all three types of anthocyanidin and with all three types of glycosides. Penetrance is complete in the *oR* class, but not in *OR* and *or* classes ; and approximately twice the amount of cyanidin is found in *oR* plants as in the others.

A survey of some hundreds of species (Lawrence *et al.*, 1939) showed that cyanidin is by far the commonest anthocyanidin in the foliage of flowering plants and it predominates in the flowers. On this evidence, and from supporting data, it was argued that whatever may be the starting point and intermediary reactions in the biosynthesis of anthocyanidins, cyanidin is the aglycone most readily produced by the plant and by the smallest number of stages. In other words, cyanidin as a final metabolic product is primitive, though not necessarily of a primitive nature (Haldane, 1941). Delphinidin and pelargonidin occur most widely in the most highly evolved plants and especially in garden plants, *i.e.* highly selected forms, and may therefore be described as the more highly evolved anthocyanidins. For the same reason, methylated anthocyanidins may be said to be more highly evolved than unmethylated.

The survey revealed that in a number of genera and species

cyanidin, in small amount, accompanies delphinidin and pelargonidin as a second pigment as if it were a biosynthetic residue or by-product. This can be seen, for example, in *rexii* and *meyeri*, but it is in the garden forms of *Streptocarpus* that it is so prominent a feature. The fact that cyanidin occurs in equal amount in *OR* and *or* classes, *OR* comprising methylated forms and *or* unmethylated (because pelargonidin is never methylated) suggests that these two classes have the same constitution with respect to *M*, hence with the *OR* class also. This view is, of course, necessitated on other grounds, *i.e.* nearly all garden forms are homozygous dominants for *M*. Linkage between *m* and *R* is thus ruled out and the preponderance of cyanidin in the *OR* class must, on the simplest view, be attributed to interaction involving *M* and *R*.

The corollary to the view that delphinidin and pelargonidin are more highly evolved pigments than cyanidin is that their synthesis involves additional processes or routes. Our data are of too general

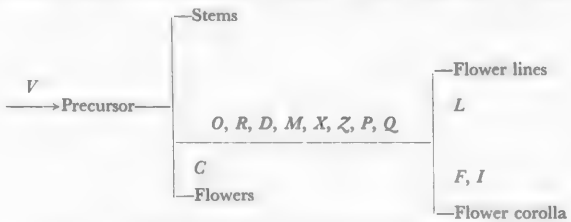


FIG. 4.—Tentative scheme for the synthesis of flower pigments in *Streptocarpus*.

a nature to warrant speculation on the various possibilities of cyanidin synthesis but they do emphasise the key position of cyanidin in the synthesis, and evolution, of the anthocyanidins.

(iv) *Pigment synthesis*

A tentative scheme for the synthesis of anthocyanins and anthoxanthins in *Streptocarpus* is given in fig. 4 based on the following considerations :—

(1) *V* controls production of a basic pigment precursor.
(2) Competition occurs in the production of chlorogenic acid and the precursor but, unlike the case with co-pigment, competition is not found between chlorogenic acid and the products of *O*, *R*, *D*, *M*, etc.
(3) *O*, *R*, *D* and *M* modify anthocyanidin in the flowers but not in the flower stems.
(4) *F* and *I* control general anthocyanidin production in the corolla but not in the throat lines.

(v) *Species*

The flowers of the great majority of *Streptocarpus* species examined are pigmented by malvidin 3 : 5-dimonoside (*ODM*) plus, in some

cases, small amounts of unmethylated pigment. Therefore there has been strong natural selection in favour of malvidin 3 : 5-dimonoside which must have a high adaptive value.

Examination of thirty-three species hybrids has revealed a strong tendency to complete dominance of *O*, *D* and *M*, i.e. of those genes with the highest frequency in the wild. Another aspect of this phenomenon concerns the dominance of *O*. This gene is a hypermorph and, further, in its presence such genes as *D*, *X*, *Z*, *M* and *P* exert their greatest effect (it is not known whether this interaction is in any sense reciprocal). *R*, another hypermorph, is hypostatic to *O*, but as it occurs in the majority of species presumably it too may have a positive adaptive value, *e.g.* it could augment the effect of *O* to give this gene a still higher "safety factor". *R*, like *O*, has the power to increase the effect of *D*, *X*, *Z* and *P*, less than does *O*, but more than does *or*.

The interaction effects found in the pigment syndrome were all observed in garden forms of *Streptocarpus*. These were derived originally from artificial crosses between *rexii*, *parviflorus* and *dunnii* :

rexii	*V*, *F*, *i*, *O*, *r*, *D*, *XxZ*—, —, *M*, *C*
parviflorus	*V*, *f*, *i*, *O*, *R*, *D*, ————, —, *M*, —
dunnii	*v* ?, *F*, *I*, *o*, *R*, *d*, *xxZZ*, *P*, *m*, *c*

The flower colour of *dunnii* is, so far, unique in *Streptocarpus* ; indeed this species exhibits a number of unusual, if not unique, characters and has a restricted habitat. From evolutionary, morphological and synthetic points of view, the flower pigment (cyanidin 3-pentoseglycoside) could be described as primitive and the adaptive value of *R* as only moderate. But *R*, in the absence of *O* in *dunnii*, has been fully exposed to the pressure of natural selection, hence it is of interest that *dunnii*, unlike *rexii*, also carries a gene *I* that increases anthocyanidin production in a marked degree. *I*, in fact, could be regarded as a modifier favouring dominance of *R* (Fisher, 1932).

These observations are based on the behaviour of *O*, *R* and *I* in the garden forms. *I* could only have been contributed by *dunnii*, but *R* might have been derived from either *parviflorus* or *dunnii*. We have seen (tables 3, 4) that *I* increases the amount of pigment in all three anthocyanidin types, even though its function in *dunnii* appears to concern cyanidin only. Therefore *I* must influence the synthesis of anthocyanidin at a point prior to that at which the specific nature of the anthocyanidins is determined. And *I* is an example of how a mutation that has arisen in one specific genetic environment may have potentialities for another, should hybridisation encourage gene migration. Speculation along similar lines can be made with reference to the evolution and action of *R*, which is associated with *d* in *dunnii*, and *D* in *parviflorus*, but interacts with both *d* and *D* in the garden forms.

(vi) *Evolution of flower colour*

Two major mechanisms concerned with floral pigmentation can be seen at work in the genus. The first maintains a very high percentage of cyanic forms in the wild population by adjustment of their nuclear and cytoplasmic balance. The second maintains a high percentage of blue-flowered species (malvidin 3 : 5-dimonoside), in which the pigment syndrome ensures a high level of pigment production and phenotypic and chemical stability.

A possible sequence of events in the course of evolution of the genes taking part in the synthesis of flower pigments in *Streptocarpus*, and other genera, can now be envisaged. The basis for a general hypothesis is as follows :

(i) Cyanidin monoside is the commonest pigment associated with the vegetative organs of flowering plants, the more complex anthocyanins being associated with the more highly evolved floral organs.

(ii) In the absence of the relevant epistatic or dominant gene, the pigment produced is less complex in structure (fewer hydroxyls, sugars), e.g. *OR* malvidin, *oR* peonidin ; *D* (or *XZ*) 3 : 5-dimonoside, *dxZ* pentoseglycoside. Thus, decreased complexity is correlated with decrease in the number of dominant and epistatic genes.

(iii) Each pigment gene that has become established in the evolutionary sequence is assumed to have a higher survival value than its predecessor and to be epistatic to it, for example : *Q* (3-monoside)→*P* (3-pentoseglycoside)→*X*, *Z* (3 : 5-dimonoside in limited amount)→*D* (3 : 5-dimonoside in large amount). Further chemico-genetical analysis might reveal that other combinations of sugar type and position exist in *Streptocarpus*, nevertheless the same evolutionary principle could still apply.

(iv) The synthesis of a new and more complex pigment in evolution requires a new and additional process. The corollary to this requirement is that the synthesis of the new pigment does not depend sequentially on the synthesis of the old one.

(v) Increase in the total number of pigment genes demands, at least to some extent, an increase in the total amount of precursor and pigment (cf. *Dahlia*) hence, a higher threshold or safety factor also.

If in general such a scheme is correct then the hypostatic genes are also relic genes ; they are milestones marking the road of evolution. That they still survive could be due to their still having a function in pigment production *e.g.* (cf. *R*, p. 321) as accessories.

6. CONCLUSION

Malvidin 3 : 5-dimonoside predominates as the main anthocyanin in the flowers of *Streptocarpus* species. It is therefore the best adapted anthocyanin. The genes mainly responsible for the determination of anthocyanidin type (*O*, *R*) are hypermorphs.

The activity of other genes taking part in anthocyanin production and modification is at a higher level in the presence of *O*, and *R* than in their absence. The genes show independent inheritance. All together, they comprise a syndrome of interacting units which promote chemical and phenotypic stability, *i.e.* homeostasis. Evolution of flower colour appears to have been by successive genes mutating, becoming dominant and finally epistatic to their predecessors.

7. SUMMARY

1. A survey is made of flower colour in the genus *Streptocarpus*, in the garden forms and in species and hybrids. Anthocyanins and anthoxanthins are identified, their inheritance demonstrated and gene action discussed.

2. The *anthocyanins* are derivatives of delphinidin, cyanidin and pelargonidin. The glycosides are 3 : 5-dimonoside, 3-pentoseglycoside and 3-monoside. The *anthoxanthins* are a co-pigment, probably apigenin, and chlorogenic acid. The phenotypic effect of the genes concerned in floral pigmentation is deduced as follows.

Gene action

3. *V* produces cyanidin glycosides in flower stems and leaves. When *F* is present also, anthocyanin is produced in the flowers, its nature depending on the presence or absence of other dominant genes.

4. In *O*(*R*), *oR* and *or* flowers the anthocyanidin types are delphinidin, cyanidin and pelargonidin respectively. *O* is epistatic to *R*. In the presence of *M*, the anthocyanins are malvidin, peonidin and pelargonidin.

5. In the presence of *D*, 3 : 5-dimonoside is produced in quantity. In *d* flowers, 3 : 5-dimonoside is produced in limited amount in the presence of the complementary genes *X* and *Z*, the accompanying pigment being 3-pentoseglycoside or 3-monoside.

6. The production of 3-pentoseglycoside and 3-monoside is postulated to be governed by other independent genes, *P* and *Q*.

7. Cyanidin is frequently a minor component in peonidin types but much less frequently and in much smaller amount in malvidin and pelargonidin types.

8. *C* controls the production of a co-pigment, probably apigenin. Chlorogenic acid occurs as a non-co-pigment in all flowers tested.

9. The inheritance of *V*, *O*, *R*, *D* and *C* is Mendelian and independent.

Varieties and species

10. In a relatively unbiased population of 133 garden forms of *Streptocarpus*, interaction was found to occur between all the pigment genes, which together constitute a syndrome promoting chemical and phenotypic stability, *i.e.* homeostasis.

11. Eighteen species tested were pigmented wholly or mainly by malvidin 3 : 5-dimonoside (*O M D*) and this anthocyanin was found to predominate in 33 crosses between species. In the wild, therefore, malvidin 3 : 5-dimonoside has a high adaptive value.

12. A high percentage of cyanic phenotypes is maintained in the wild population by adjustment of the nuclear and cytoplasmic balance of the species.

13. The hypothesis is advanced that the evolution of flower colour in *Streptocarpus* has been by successive genes mutating, becoming dominant and, finally, epistatic to their predecessors.

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APPENDIX

Identification of pigments

Anthocyanins were fully identified by means of the qualitative tests devised by Robinson and Robinson, 1931, 1932.

The glycoside types were distinguished by their distribution to *iso*-amyl alcohol. At low concentrations monosides are extracted by the organic layer up to 30 to 40 per cent. (according to the degree of oxidation of the anthocyanidin, pelargonidin derivatives having the highest distribution) pentoseglycosides are extracted scarcely at all and dimonosides not at all. If the solutions are then saturated with salt the monosides and pentoseglycosides are both completely extracted but the distribution of the dimonosides is unchanged.

For complete identification of the anthocyanin pigments, flowers, after removal of corolla tubes, were stored in bottles in 1 per cent. aqueous HCl. The crude extract was filtered and tested for co-pigment and other anthoxanthins. If present, these were removed by repeated extraction with ethyl acetate. The solution was finally washed with benzene and filtered.

A portion of the purified extract was saturated with salt and shaken with an equal volume of amyl alcohol. If no pigment was extracted by the acohol the anthocyanin was diglycosidic and was then hydrolysed straight away. If, however, some or all of the pigment passed into the alcohol layer it was necessary to separate the glycosidal types by saturating the whole of the solution with salt and extracting exhaustively with amyl alcohol. The combined amyl alcohol extracts were filtered and the pigment returned to 1 per cent. aqueous HCl by the addition of much light petroleum. By this means, the pigments with high distribution were separated from the dimonosides. Each fraction was examined separately, the glycosidal types identified and the approximate percentage of the whole which each fraction represented estimated by colorimetric comparison of the 1 per cent. aqueous HCl solutions.

The presence of 3-monosides in *Streptocarpus* was demonstrated by comparing colorimetrically the distribution to *iso*-amyl alcohol of the purified high distribution fraction from a rose-coloured flower in 1 per cent. aqueous HCl, with the distributions given by comparable solutions of pure crystalline 3-monosides of cyanidin and malvidin. (Results : cyanidin 3-monoside 29·8 per cent., malvidin 3-monoside 22·9 per cent. and high distribution fraction from rose-coloured flower 25·7 per cent.).

The anthocyanidins obtained following the hydrolysis of the glycosides were identified by Robinson and Robinson's aglycones qualitative tests. In many *Streptocarpus* garden forms there is a mixture of anthocyanidins as well as sugar types. These were separated by making use of the different distributions displayed by the anthocyanidins to the " cyanidin reagent " and " delphinidin reagent " of Robinson and Robinson. A complete separation cannot always be achieved but cyanidin, which is the unmethylated type most frequently found in the garden forms, can always be identified as it is the only pigment extracted by " cyanidin reagent " that gives a positive ferric reaction.

STUDIES ON *STREPTOCARPUS*

IV. GENETICS OF FLOWER COLOUR PATTERNS

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1. INTRODUCTION

In a previous paper (Lawrence and Sturgess, 1957) data were given for the chemical constitution and inheritance of the main anthocyanin pigments found in flowers of garden forms of *Streptocarpus* ($2n = 32$) and in the species and their hybrids.

It was shown that *V* genotypes have anthocyanin in their flower stems (and to a lesser extent in the leaves) but not in their flowers; *vF* genotypes have anthocyanin in neither their stems nor their flowers; while *VF* genotypes have pale anthocyanin in their flowers as well as pigmented stems. Consequently *V* and *F* act as complementary genes governing the production of anthocyanin in the flowers. A third gene, *I*, increases the amount of anthocyanin in the flowers, giving colour of medium to deep intensity in place of pale, *i*. *V*, *F* and *I* control the production of anthocyanin in the flowers apparently irrespective of the nature of the pigment, while the production of specific anthocyanins is determined by alleles *Oo*, *Rr* and *Dd* which, in their appropriate combinations, give malvidin, peonidin, and pelargonidin glycosides.

2. PATTERN IN THE GARDEN FORMS

In this paper, data are given for the inheritance of four flower colour patterns.

(i) anthocyanin blotch (*B*) within the corolla tube.
(ii) anthocyanin (*H*) in capitate hairs on the pistil.
(iii) anthocyanin lines (*L*) on the petals.
(iv) yellow pigment (*Y*) in a central stripe down the corolla tube.

In a given genotype, the main pigment in the lines has been identified as being the same as that in the petals.

Table 1 gives the genotypes for some contrasted phenotypes. It should be noted that the occurrence of coloured capitate hairs (*H*) and of lines (*L*) is conditional upon the presence of *V*; and the occurrence of blotch (*B*) upon the presence of *V* and *F*.

(*a*) *Inheritance of* F, I, B *and* H

The detailed phenotypic effect of these genes in the presence of *V* is as follows; *F* produces anthocyanin uniformly in the epidermal cells of the whole corolla. *I* intensifies this colour. *B* produces a

blotch of deeper anthocyanin on the anterior portion of the inside of the corolla tube around the point of insertion of the anther filaments.

TABLE 1

Distribution of anthocyanin in the plant

Genotype	Phenotype				
	Corolla (*F*)	Corolla tube blotch (*B*)	Pistil hairs (*H*)	Lines (*L*)	Flower stems and foliage
vfi bhl	–	–	–	–	–
vFi BHL	–	–	–	–	–
Vfi bhl	–	–	–	–	+
Vfi bHL	–	–	+	+	+
VFi bhl	+	–	–	–	+
VFi BHL	+	–	+	+	+
VFI bhl	++	–	–	–	+
VFI BHL	++	++?	++?	++?	+

H produces anthocyanin in the stalks of the glandular hairs on the pistil but not in the intermingled eglandular hairs.

The inheritance of *F* has been given previously (Lawrence and Sturgess, 1957). The breeding results were consistent with respect

TABLE 2

Inheritance of blotch (B) *and hair colour* (H)

Genes	No. of families	*X* : *x*	χ^2		D.F.	*P*
			Deviation from	Hetero-geneity		
		B1	*1* : *1*			
Bb	25	585 : 542	1·641	...	1	0·20
				21·402	24	0·7-0·5
Hh	17	429 : 374	3·767	...	1	0·1-0·05
				13·152	16	0·7-0·5
		F2	*3* : *1*			
Bb	24	1006 : 350	0·164	...	1	0·7-0·5
				19·118	23	0·7
Hh (*FF* plants only)	20	862 : 251	3·690	...	1	0·1-0·05
				13·872	19	0·8-0·7

to the expected genotypes, but in the majority of families there was a deficiency of *F* individuals. The inheritance of *I* was also given and was straightforward. The inheritance of *B* and *H*, separately, is shown in table 2, and is, basically, monohybrid. The expected *B*, *b* phenotypes always occur but in some cases, omitted from the summarised data in the table and to be discussed later, there are significant deviations from expectation. A similar situation is found

with respect to *H*, *h* phenotypes in families homozygous for *F*, table 2. In families segregating for *F*, 64 per cent. show a marked deficiency of *Fh* genotypes. We may summarise the data so far presented by saying that the inheritance of all four dominant genes, considered separately, is monohybrid, but often there is a deficiency of cyanic individuals in families segregating for both *F* and *H*.

The reason for these deficiencies becomes clear when the data are examined for the combined inheritance of *F*, *I*, *B* and *H*. These "genes" are then seen to behave as if they occur very nearly at the same locus and comprise a complex or supergene, a postulation which will be examined in detail later. Throughout this paper the terms gene and allele will be used to describe the components of the supergene, without prejudice to their precise nature, location or action.

TABLE 3

Garden form genotypes

Class	1	2	3	4	5	6	7
Genotype	$\frac{FiBH\ (r)}{FiBH\ (r)}$*	$\frac{FiBH\ (r)}{FIbh\ (d)}$	$\frac{FIbh\ (d)}{FIbh\ (d)}$	$\frac{FiBH\ (r)}{fibH\ (p)}$	$\frac{FIbh\ (d)}{fibH\ (p)}$	$\frac{fibH\ (p)}{fibH\ (p)}$	$\frac{FIbH}{fibH}$
No. of plants identified	13	16	65	22	28	6	3

* ancestral gametes : *r* = *rexii*, *d* = *dunnii*, *p* = *parviflorus*.

(i) *Evidence from the parents.* *F*, *I*, *B* and *H* genotypes have been identified in 153 garden parents. These fall into only 7 classes instead of the 81 expected from the independent inheritance of four pairs of alleles (table 3).

With the exception of the three class 7 individuals, these results can be interpreted on the basis of complete linkage, the six genotypic classes being derived from the combinations of three types of gamete, viz. *FiBH*, *FIbh* and *fibH*. These six classes are readily identified by sight in respect of *F*, *B* and *H* but the cumulative effects of both *F* and *I* often prevent reliable identification of *I* phenotypes. However, once a genotype has been determined in respect of the other three genes, its constitution for *I* can be inferred on the basis of complete linkage, pedigrees can be examined, and proof obtained by breeding. This has been done in many cases.

The sequence of the four pairs of alleles on the chromosome is not known. The order in which they are given is based on the extent (area) of their action : *F* and *I* are concerned with the pigmentation of the whole flower whereas *B* and *H* are concerned with highly limited areas.

We have now to find an explanation for the occurrence of the three exceptional class 7 genotypes. Their parents and progeny are

TABLE 4

Origin, constitution and progeny of the anomalous types $6^{6}/34$, $4^{7}/36$ and $69^{19}/36$

Parents		Progeny			
				Number expected	
Female	Male	Expected types	Number observed	Full viability	Zygotic lethality
$3\ 33\ \frac{FIbh}{FiBH}$	$1/33\frac{fibH}{fibH}$	$6/34\ \frac{FIbh}{fibH}$	45	49·5	49·5
		$\frac{FiBH}{fibH}$	54	49·5	49·5
$6^{6}/34\ \frac{FIbH^{*}}{fibH}$ (mutant)	selfed	$14/35\ \frac{FIbH}{FIbH}$	0	18·5	lethal
		$\frac{FIbH}{fibH}$	43	37·0	49·3
		$\frac{fibH}{fibH}$	31	18·5	24·7
$1/33\ \frac{fibH}{fibH}$	$6^{6}/34\ \frac{FIbH}{fibH}$	$25/35\ \frac{fibH}{FIbH}$	35	35·5	35·5
		$\frac{fibH}{fibH}$	36	35·5	35·5
$15\ 34\ \frac{FIbh}{FiBH}$	$14^{4}/34\ \frac{fibH}{FIbh}$	$4/36\ \frac{FIbh}{fibH}$	51	39·0	39·0
		$\frac{FIbh}{FIbh}$	24	39·0	39·0
		$\frac{FiBH}{fibH}$	81	78·0	78·0
		$\frac{FiBH}{FIbh}$			
$4^{7}/36\ \frac{FIbH^{*}}{fibH}$ (mutant)	selfed	$92/37\ \frac{FIbH}{FIbH}$	0	12·5	lethal
		$\frac{FIbH}{fibH}$	28	25·0	33·3
		$\frac{fibH}{fibH}$	22	12·5	16·7
$28^{62}\ 35\ \frac{fibH}{FIbh}$	selfed	$69/36\ \frac{fibH}{fibH}$	8	11·75	11·75
		$\frac{fibH}{FIbh}$	39	35·25	35·25
		$\frac{FIbh}{FIbh}$			
$69^{19}/36\ \frac{fibH}{FIbH^{*}}$ (mutant)	selfed	$82/38\ \frac{fibH}{fibH}$	9	7·25	9·7
		$\frac{fibH}{FIbH}$	20	14·50	19·3
		$\frac{FIbH}{FIbH}$	0	7·25	lethal
$4^{24}/36\ \frac{FiBH}{fibH}$	$69^{19}/36\ \frac{fibH}{FIbH}$	$285/39\ \frac{FiBH}{fibH}$	13	12·5	16·7
		$\frac{FiBH}{FIbH}$	0	12·5	lethal
		$\frac{fibH}{fibH}$	21	12·5	16·7
		$\frac{fibH}{FIbH}$	16	12·5	16·7

* = mutant gametes.

given in table 4. First, it will be seen that, whereas from their parentage these individuals should have been heterozygous for *H*, all of them bred as if they were homozygous dominants. Secondly, on the basis of the known viable gametes and zygotes (table 3), and on the breeding results (table 4) all three of the class 7 genotypes must be allotted the constitution $\frac{FIbH}{fibh}$ in which the *FIbH* gamete is novel. Thirdly, the kinds and proportions of the cyanic phenotypes in test crosses, especially in family 25/35, show that the gamete *FIbH* is viable. Fourthly, the ratios obtained in families 14/35, 92/37, 82/38 and 285/39 indicate that the zygotes $\frac{FIbH}{FIbH}$ and $\frac{FIbH}{FiBH}$ carrying the *FIbH* gamete are lethal. Lastly, it will be seen that the *FIbH* gamete could have arisen by (1) crossing-over between *H* and *h*, (2) simultaneous crossing-over between *iB* and *Ib*, or (3) by mutation of *h* to *H*, or of *iB* to *Ib*. Whichever event was effective, the specificity and independence of the relevant loci is demonstrated.

(ii) *Evidence from the progeny.* The recording of the flower pattern characters dealt with in this paper was originally ancillary to the main study of the genetics and chemical constitution of the anthocyanin pigments, hence the data presented in this section are merely those that happened to come to hand. Nevertheless, they support and amplify the conclusions derived from the genotypic constitution of the parents discussed in the previous section. Table 5 gives the summarised results for linkage between the components of the supergene. Except, apparently, in two reciprocal families, 102/38 and 103/38, linkage is complete. The occurrence of these exceptions is of much interest since they directly support the view that the individuals $6^{6}/34$, $4^{7}/36$ and $69^{19}/36$ arose by crossing-over or mutation at either one " locus " or two. In the case of these three individuals mutation, in the broad sense, was from the heterozygous to the dominant homozygous phase, therefore it was not directly observable and could only be deduced by the breeding behaviour of the parents. In the case of families 102/38 and 103/38, three mutant phenotypes were directly observed. The details of their parents and progeny are given in table 6 and they show that the genotypes of the three mutants are identical. There appears to be only one explanation of their origin that fits all the facts, namely, that the *i* locus in the white-flowered parent $1/33\ \frac{fibH}{fibH}$ mutated to *I* giving the mutant genotype $\frac{f\,IbH}{FiBH}$. Crossing-over, in the superficial sense, is therefore excluded in this instance.

A further instance of crossing-over or mutation is provided by a back-cross family 14/54 ((*rexii* $FB \times 14^{1}/34) \times 14^{1}/34$), table 7. The constitution of the parents $1^{21}/53$ and $14^{1}/34$ is known from a number of selfs and crosses in which they have been used. In family 14/54

TABLE 5

Linkage summary

Combination	Families	*XY*	*Xy*	*xY*	*xy*	Expectation with complete linkage	Cross-over percentage and standard error
$\frac{FB}{fb}$ selfed	1	38	0	0	16	3 : 0 : 0 : 1	0
$\frac{BH}{bh}$ selfed	25	1095	0	0	331	3 : 0 : 0 : 1	0
$\frac{BH}{bh} \times \frac{bh}{bh}$	12	292	0	0	240	1 : 0 : 0 : 1	0
$\frac{BH}{bh} \times \frac{bH}{bh}$	4	154	0	114	48	2 : 0 : 1 : 1	0
$\frac{Fh}{fH}$ selfed	5	124	56	62	0	2 : 1 : 1 : 0	0
$\frac{Bi}{bI}$ selfed	2	110	64	43	0	2 : 1 : 1 : 0	0
$\frac{Bi}{bI} \times \frac{bi}{bi}$	4	3*	126	123	0	0 : 1 : 1 : 0	...
$\frac{Hc}{hC} \times \frac{hc}{hc}$	1	6	9	9	6	...	40·0±8·9
$\frac{Bc}{bC} \times \frac{bc}{bc}$	1	6	20	17	7	...	26·0±6·2
$\frac{Bc}{bC} \times \frac{Bc}{bc}$	1	12	4	14	3	...	42·0±15·7†
$\frac{Cy_2}{cY_2}$ selfed	2	47	29	22	0	2 : 1 : 1 : 0	0
$\frac{Y_2D}{y_2d}$ selfed	1	50	16	8	30	...	20·2
$\frac{Y_1D}{y_1d}$ selfed	1	22	5	16	12	...	34·4
$\frac{Y_1D}{y_1d} \times \frac{y_1d}{y_1d}$	2	34	13	13	37	...	26·8± 4·5 } 25·3± 4·2
$\frac{Y_1D}{y_1d} \times \frac{y_1D}{y_1d}$	1	16	1	12	9	...	12·3±10·2 }
$\frac{Dl}{dL}$ selfed	2	85	19	55	0	...	} 22·8± 2·7 ‡
$\frac{Dl}{dL} \times \frac{dl}{dl}$	7	56	124	164	33	...	}

* = mutants, see families 102-3/38, table 6.
† = by maximum likelihood.
‡ = based on a differential survival rate of *ll* genotypes estimated as 64·5±6·1 per cent.

TABLE 6

Origin, constitution and progeny of anomalous types 102[22]/38, 103[8]/38 and 103[40]/38

Parents		Progeny		
Female	Male	Expected types	Number observed	Number expected
1′33 $\frac{fibH}{fibH}$	16/34 $\frac{FiBH}{Fibh}$	102/38 $\frac{fibH}{FiBH}$ and 103/38 $\frac{fibH}{FIbh}$	45	45
		$\frac{fIbH}{FiBH}$	45	45
			3*	...
$102^{22}/38\frac{fIbH^*}{FiBH}$ (mutant)	selfed	259/39 $\frac{fIbH}{fIbH}$	15	11·5
		$\frac{fIbH}{FiBH}$ $\frac{FiBH}{FiBH}$	31	34·5
116^{47} 38 $\frac{FIbh}{FIbh}$	$102^{22}/38\frac{fIbH^*}{FiBH}$	262/39 $\frac{FIbh}{fIbH}$	30	22·5
		$\frac{FIbh}{FiBH}$	15	22·5
$103^{8}/38\frac{fIbH^*}{FiBH}$ (mutant)	selfed	260/39 $\frac{fIbH}{fIbH}$	11	12·5
		$\frac{fIbH}{FiBH}$ $\frac{FiBH}{FiBH}$	39	37·5
$103^{40}/38\frac{fIbH^*}{FiBH}$ (mutant)	selfed	261/39 $\frac{fIbH}{fIbH}$	12	12·25
		$\frac{fIbH}{FiBH}$ $\frac{FiBH}{FiBH}$	37	37·75

* = mutant gametes.

two plants appeared having a totally new combination of the four genes, viz. *FibH*. There are four possibilities for the origin of this combination, (1) crossing-over between $\frac{B}{b}$ in $1^{21}/53$ to give the gamete *FibH*, (2) similar crossing-over between $\frac{F}{f}$ in $1^{21}/53$, (3) crossing-over between $\frac{f}{F}$ in $14^{1}/34$ and (4) crossing-over between $\frac{i}{I}$ in $14^{1}/34$. Alternatively, mutation of *B* to *b* or *f* to *F* in $1^{21}/53$; or of *f* to *F* or *I* to *i* in $14^{1}/34$, would give the same end result as crossing-over. Incidentally the deficiency of $\frac{FiBH}{FIbh}$ individuals is paralleled in other families in which this genotype segregates.

TABLE 7

Origin of anomalous segregates

Parent		Progeny		
Female	Male	Expected types	Number observed	Number expected
$1^{21}/53\ \frac{FiBH}{fibH}$	$14^{1}/34\ \frac{fibH}{FIbh}$	$14/54\ \frac{FiBH}{fibH}$	11	9·5
		$\frac{FiBH}{FIbh}$	3	9·5
		$\frac{fibH}{fibH}$	12	9·5
		$\frac{fibH}{FIbh}$	12	9·5
		$\frac{FibH}{—ib—}$	2 (mutant)	...

We can now consider the collective evidence provided by parental genotypes and progeny phenotypes and their ratios. Three identical mutants have been identified among garden parents (table 4), three in the progeny of one garden cross (table 6), and two probably identical mutants in a derivative of a cross between a species and garden form (table 7). The mutant gametes were *FIbH*, *fIbH* and *FibH* respectively. In the second of the above categories, gene mutation of *i* to *I* appears to have occurred. In the other two categories the possibilities of crossing-over or mutation are various. In any case, the evidence suggests that *F*, *I*, *B* and *H* are specific units of a complex gene, each with independent phenotypic effects.

(iii) *Lethality*. The limited number of gametes determining flower pattern in garden forms of *Streptocarpus* requires further consideration. All the material employed in these studies originated from six plants

(the gametic pool) chosen for their distinctive flower colours from Messrs Peed's strain of *Streptocarpus* :

1/33, white $\frac{fibH}{fibH}$; 2/33, blue $\frac{FIbh}{FIbh}$; 3/33, rose $\frac{FIbh}{FiBH}$;

15/34, salmon $\frac{FIbH}{FiBh}$; 16/34, magenta $\frac{FIbh}{FiBH}$; 18/36, petunia $\frac{FIbh}{FIbh}$.

Six other plants of Peed's strain were obtained later from a different source, but these proved the same, genotypically, as one or other of the first lot, so they were not used in the main breeding programme.

As will be seen by reference to table 3, the gametic pool contains all the types identified (excluding the mutants) and no others. Because the pool is a small one, the existence of other types of gametes in garden *Streptocarpus* cannot be precluded, although perhaps it is unlikely. But the assumption that there are, apart from the mutants observed in these experiments, only three types of viable gamete in garden *Streptocarpus* assumes considerable significance when the constitution of the three species which were the parents (Lawrence and Sturgess, 1951) of the garden forms is considered, viz. *rexii*, *FiBH* ; *dunnii*, *FIbh* ; *parviflorus*, *fibH*, i.e. precisely the same as those of the only three types of gametes found in the garden forms. The corollary of this assumption is that all, or nearly all, other gametes are lethal in the garden forms. (Other combinations of the supergene components occur in other species.) Each parental combination of the supergene is balanced, recombination resulting in gametic lethality.

Further light is thrown on gametic behaviour by noting the inheritance of *H* and *h*. When $\frac{FIbh}{FiBH}$ plants were selfed, the ratios of *H* to *h* were good 3 : 1's (table 2), but when $\frac{FIbh}{fibH}$ plants were selfed, the ratios were abnormal, the total numbers from 25 different families being *Fh* 263 ; *FH* 912 ; *f H* 637, *i.e.* approximately 3 : 10 : 7 when expectation was 1 : 2 : 1. This result cannot be explained merely by supposing that some of the $\frac{FIbh}{FIbh}$ zygotes were lethal as this would not account for the excess numbers of the *f H* class. On the other hand, a fair approximation to the actual F_2 ratio is got if we deduce coefficients of " viability " such that *fibH* = 1·0 and *FIbh* = 0·64.

Applied to the gametes only, the coefficients could be a measure of certation, e.g. *fibH* pollen-tubes grow more quickly down the styles than *FIbh* and, on the average, compete successfully in fertilisation in the ratio *ca.* 1·0 : 0·64. On the other hand, the product coefficients could be a measure of average zygotic viability. Large differences in vegetative vigour occur among the garden *Streptocarpus* ranging from luxuriant growth to sub-lethality, *e.g.* inability to survive under

excellent conditions of cultivation for more than a few months. Clearly it is a small step from this sub-lethality, to lethality at some point between fertilisation and seed germination, the evidence for which we shall now examine.

Firstly, examples of zygotic lethality are seen in families 14/35, 92/37 and 82/38 (table 4) in which no $\frac{FIbH}{FIbH}$ mutant genotypes occurred when the expected numbers were 18·5, 12·5 and 7·25 respectively.

TABLE 8

Differential viability of $\frac{\text{FIbh}}{\text{FIbh}}$ *zygotes*

Parents		Progeny			
Female	Male	Expected types	Number observed	Number expected	
				Full viability	Zygotic lethality
$1^6/53\ \frac{FiBH}{FIbh}$	$14^1/34\ \frac{fibH}{FIbh}$	$11/54\ \frac{FiBH}{fibH}$	18	9·5	12·7
		$\frac{FiBH}{FIbh}$	11	9·5	12·7
		$\frac{FIbh}{fibh}$	9	9·5	12·7
		$\frac{FIbh}{FIbh}$	0	9·5	0
$1^6/53\ \frac{FiBH}{FIbh}$	selfed	$21/54\ \frac{FiBH}{FiBH}$	11	8·0	10·7
		$\frac{FiBH}{FIbh}$	20	16·0	21·3
		$\frac{FIbh}{FIbh}$	1	8·0	0

The *FIbH* gamete must be assumed to be viable since, in the same families, it functioned to give reasonably good ratios in combination with *fibH* and *FIbh* gametes, hence it was the homozygote $\frac{FIbH}{FIbH}$ that was lethal. Secondly, in family 285/39 (table 4) there were no $\frac{FiBH}{FIbH}$ individuals when the expected number was 12·5. Thus the *FIbH* gamete appears to be lethal in some zygotic combinations and viable in others. Thirdly, no $\frac{FIbh}{FIbh}$ progeny were found in family 11/54 (table 8) when the expected number was 9·5 ; and in family 21/54 there was only one member of this class when expectation was 8·0. Since 65 garden forms have been found to have the constitution $\frac{FIbh}{FIbh}$,

differential zygotic lethality must be postulated to account for this and the other results mentioned above.

The results of the experiments on the above four pairs of factors concerned with flower pattern in the garden forms may be summarised as follows :—

(1) *F*, *I*, *B* and *H* and their recessives are components of a supergene.

(2) With very rare exceptions the only viable gametes are those in which the components are identical with those of the three species known to be the parents of the garden forms.

(3) Certain combinations of the parental types of gamete result in zygotic sub-lethality.

(4) Three types of mutant gametes comprising new combinations of the components of the supergene have proved to be viable. Under glasshouse conditions, one mutant is lethal in the homozygous phase and with two of the three parental gametes, but viable with the third. The second mutant is viable in the homozygous phase and with the two parental gametes tested. The third mutant was not tested.

(*b*) *Inheritance of* L

Narrow lines of anthocyanin are commonly found on the three posterior petals of both cyanic and acyanic flowers of *Streptocarpus*. These lines start just outside the throat of the flower and run down the corolla tube half-way or more. They may be continuous or somewhat broken. A common pattern is 2—3—2 with respect to the number of lines found on the three lower petals. In acyanic (*Vf*) flowers the lines are very conspicuous ; in cyanic flowers they are more deeply coloured than the petals and vary from being well-marked to barely discernible. In some individuals the middle posterior petal is without lines and those on the adjacent petals are broken and somewhat diffused, forming " side-bands ". Both the 2—3—2 and side-band patterns are controlled by the *L* gene, the differences in pattern presumably being due to modifying genes.

The summarised results for the inheritance of *L* in acyanic garden forms are given in table 9. In B_1 and F_2 agreement with expectation is satisfactory in acyanic forms and the data are homogeneous. In cyanic forms recessives are deficient in number, the F_2 ratio being 6·35 *L* : 1·0 *l* and B_1 1·54 *L* : 1·0 *l*. These ratios are of the order that would be expected if a common gametic factor is operating, *e.g.* if *l* gametes participate in fertilisation less frequently than *L* gametes, in the ratio *ca.* 1·0 *L* : 0·61 *l*. The similarity of this ratio to the coefficient of 0·64 for *FIbh* participation in fertilisation (p. 345) is of note. Examination of all relevant data has failed to reveal any specific connection between the deficiency of *ll* and *FIbh* genotypes. The origin of *l* is obscure, *e.g.* the ancestral species are all *L*. Probably, it is a mutant that has arisen under cultivation and which shows differential viability in *F* genotypes.

(c) *Inheritance of* Y

A central yellow stripe, $\frac{1}{16}$ to $\frac{1}{4}$ inch wide, runs from the limb down the posterior side of the corolla tube in many garden *Streptocarpus*.

TABLE 9

Inheritance of Ll *in acyanic garden forms*

No. of families	$X : x$	χ^2		D.F.	P
		Deviation from $X : x$	Heterogeneity		
8	*B1* 115 : 137	*1 : 1* 1·921	...	1	0·5-0·1
			13·215	7	0·1-0·05
12	*F2* 198 : 61	*3 : 1* 0·072	...	1	0·8-0·7
			13·215	11	0·3-0·2

TABLE 10

Inheritance of yellow stripe, Y_1Y_2 *in garden forms*

Genes	No. of families	$X : x$	χ^2		D.F.	P
			Deviation from $X : x$	Hetero-geneity		
		B1	*1 : 1*			
Y1y1	5	230 : 258	1·607	...	1	0·3-0·2
				2·366	4	0·7-0·5
Y2y2	4	315 : 306	0·130	...	1	0·8-0·7
				0·487	3	0·9
		F2	*3 : 1*			
Y1y1	3	47 : 46	29·680	...	1	v. small
				1·320	2	0·7-0·5
Y2y2	24	928 : 360	6·146	...	1	0·02-0·01
				29·821	23	0·2
			9 : 7			
Y1y1Y2y2	4	186 : 176	3·487	...	1	0·1-0·05
				0·971	3	0·9-0·8

This character is controlled by two dominant complementary genes, *Y1* and *Y2*, such that *Y1*(*Y1*)*y2y2* and *y1y1Y2*(*Y2*) phenotypes lack the yellow stripe, whereas *Y1*(*Y1*)*Y2*(*Y2*) has it. The breeding results (table 10) are in good agreement with expectation in backcrosses but, as with *F*, in F_2 the class totals are only moderately good and total and family heterogeneity values are disturbed.

(*d*) *Linkage*

No systematic attempt was made to determine linkages of flower pattern genes and the results given in table 5 are merely those that came to hand. Linkage of *F*, *I*, *B* and *H* has been discussed and is complete, or almost entirely so. The other pattern genes, *L*, *Y1* and

TABLE 11

Inheritance of Ll *in species × garden forms*

Species	No. of families	*X* : *x*	χ^2		D.F.	*P*
			Deviation from	Hetero-geneity		
		B1	*1* : *1*			
rexii	19	389 : 303	12·874	...	1	0·001
				28·299	18	0·1-0·05
rexii FB	6	133 : 95	6·333	...	1	0·02-0·01
				8·715	5	0·2-0·1
dunnii	5	64 : 86	3·227	...	1	0·1-0·05
				8·921	4	0·1-0·05
gardeni	1	13 : 15	0·143	...	1	0·8-0·7
cyaneus	1	20 : 16	0·444	...	1	0·7-0·5
		F2	*3* : *1*			
(*a*) *rexii*	10	341 : 99	1·467	...	1	0·3-0·2
				3·502	9	0·95-0·9
(*b*) *rexii*	3	124 : 23	1·715	...	1	0·2-0·1
				5·652	2	0·1-0·05
(*c*) *rexii*	2	64 : 18	0·407	...	1	0·7-0·5
				0·260	1	0·7-0·5
rexii	2	138 : 42	0·267	...	1	0·7-0·5
				3·560	1	0·1-0·05
rexii FB	9	206 : 42	8·602	...	1	0·01-0·001
				4·428	8	0·9-0·8
		F2	*3* : *1*			
gardeni	1	36 : 19	2·673	...	1	0·2-0·1
cyaneus	5	110 : 34	0·148	...	1	0·7
				7·727	4	0·2-0·1
meyeri	1	38 : 9	0·858	...	1	0·5-0·3

(*a*), (*b*) and (*c*) = crosses with different test plants.

Y2 also exhibit linkage, crossing-over apparently being of the order 20-30 per cent. *L* is linked with *D* but not with *Y2* (*B1* = 20 *LY* : 32 *Ly* : 23 *lY* : 23 *ly*). *L*, therefore, should show linkage with *Y1* but no data exist to test this. Only tentative assumptions can be made about the linear order of the genes on the chromosome which, with approximate cross-over values, seems to be as follows :—

FIBH 30? *CY2* 20 *D* 25 *Y1* (*L*?)

The inheritance of *L* is independent of that of *O*, *F*, *B* and *H* and the inheritance of *Y1* and *Y2* independent of that of *O* and *R* (Lawrence and Sturgess, 1957).

3. PATTERN IN THE SPECIES

General anthocyanin

Evidence for the Mendelian inheritance of *V*, *F* and *I* in families derived from crosses between *rexii* and *rexii FB* on the one hand and

TABLE 12

Inheritance of yellow stripe in interspecific crosses

Combination	Excluding *rexii* and its varieties		*rexii*, *rexii L* and *insignis*	
	No. of crosses	*F1*	No. of crosses	*F1*
Yellow × yellow	31	Yellow	...	...
Non-yellow × non-yellow	42	Non-yellow	1	Yellow
			21	Non-yellow
Yellow × non-yellow (and reciprocal)	30	Yellow	16	Yellow
			7	Non-yellow

TABLE 13

Constitution for Yy *of some non-yellow species*

Species (non-yellow)	*F1* phenotypes from crossing with :				Postulated constitution of species
	Y1Y1y2y2		*y1y1Y2Y2*		
	Yellow	Non-yellow	Yellow	Non-yellow	
dunnii	0	51	0	4	*y1y1y2y2*
wendlandii	0	74	0	15	*y1y1y2y2*
grandis	15	0	...	...	(*y1y1*) *Y2Y2*
grandis albus	43	0	...	...	(*y1y1*) *Y2Y2*
rexii FB	*F1* 40	39	...	...	*y1y1Y2Y2*
	B1 73	115	...	...	
pusillus	2	0	...	...	(*y1y1*) *Y2*—
(yellow) *galpinii*	...	...	6	2	*Y1y1Y2Y2*

Brackets = inferred constitution.

garden forms on the other, was presented in Lawrence and Sturgess, 1957, page 305 and earlier in this paper (tables 7 and 8). In addition, three F_1 individuals with cyanic flower stems, derived from *rexii* (cyanic stems, *VV*) × *dunnii* (acyanic stems, *vv*) backcrossed to *dunnii*, gave a total of 178 plants with cyanic and 47 with acyanic stems (χ^2 ; 3 : 1 ratio, P = 0·05-0·1). Clearly, there is either a large deficiency of recessives in the backcross, or at least one other pair of alleles is involved. In the latter event, a 3 : 1 ratio would be obtained in B_1 if *rexii* were *VVWW* and *dunnii vvww*, *V* and *W* being supplementary genes producing anthocyanin in the stems.

Evidence for the constitution of the three species parents of the garden forms is as follows. With *rexii*, $\frac{FiBH}{FiBH}$ backcross ratios were obtained of 83 *B* : 63 *b* and 47 *H* : 38 *h* and an F_2 of 58 *H* : 17 *h*. Two $\frac{FIbh}{FIbh}$ garden forms × *dunnii* gave 42 and 30 F_1 plants respectively, all *bh*. *dunnii* lacks the corolla tube blotch (*B*) and coloured capitate hairs (*H*) on the pistil, and may therefore be assigned the constitution $\frac{FIbh}{FIbh}$. Crossed to a *bh* garden form, *parviflorus* gave 30 F_1 plants, all *bH* ; therefore this species is *fibH*.

(*a*) *Lines*

The expression of the line character, *L*, in species crosses is straightforward : 64 $L \times L$ families and 33 $L \times l$ were all lined and 21 $l \times l$ all unlined. A summary for the inheritance of *L* in 5 distinctive species is given in table 11. Often there is a deficiency of recessives ; nevertheless it is highly probable that inheritance of the line pattern is governed by a single gene.

Although the data are not sufficiently extensive to permit a firm conclusion, it is probable that (1) the anthocyanin patterns as found in the species are controlled by genes *B*, *H* and *L* homologous to those studied comprehensively in the garden forms, and (2) the inheritance of these genes is monohybrid as in the garden forms.

(*b*) *Yellow stripe*

The position regarding the complementary genes *Y1*, *Y2* for yellow throat is more complex :—

(1) Excluding crosses with *rexii*, yellow was inherited as if it were a simple dominant over non-yellow (table 12), but with varying degrees of expression.

(2) In crosses involving *rexii*, *rexii L* and *insignis*, yellow crossed non-yellow gave 16 yellow F_1's and 7 non-yellow (*rexii* itself, 9 yellow and 4 non-yellow).

(3) The F_1's from crossing *rexii*, *rexii L* and *insignis* with garden forms of various constitutions for *Y* were variable, but in general yellow was largely or totally suppressed. Indeed, sometimes the yellow pattern was not recovered in B_1 and F_2 families numbering 50-70 individuals. This is what might be expected if *rexii* etc., carried a suppressor of yellow along with some recessive *y* genes.

(4) Species lacking the yellow pattern may carry *Y1* or, alternatively, *Y2*. This has been shown by crossing such species to *y1y1Y2Y2* and *Y1Y1y2y2* garden forms, respectively (table 13). Although a complete investigation of the constitution of *rexii* was not possible, the evidence from *B2*

suggests that this species does in fact carry Y genes, and is probably $--Y_2Y_2$. This assumption accords with F_1 and B_1 evidence from the acyanic *rexii FB*.

The general conclusion, therefore, is that yellow behaves as a simple F_1 dominant in all species crosses excepting *rexii* and the closely related *rexii L* and *insignis*, these species being characterised by an hereditary factor which tends strongly to suppress yellow.

(c) *Dunnione*

This is a colouring matter, β-naphthaquinone, which occurs as an orange-red deposit on the leaves and inflorescences, but not the flowers, of *dunnii* (Price and Robinson, 1940). The same pigment, apparently, is also found on *pole-evansii*. In *rexii* × *dunnii* F_1's dunnione is completely recessive. Backcrosses to *dunnii* gave a total of 63 unpigmented : 83 pigmented plants ($\chi^2 1 : 1$, P = 0·1; Het. χ^2P = 0·07) and inheritance is, therefore, monohybrid.

(d) *Filament colour*

The anther filaments in all of the species examined, save three, are pure white or white faintly tinged with purple anthocyanin (*polyanthus*, *johannis*). The three exceptions are *michelmorei* and *eylesii* which have deep purple filaments and *dunnii* which has green, *i.e.* pigmented with chlorophyll. In four different F_1's, *michelmorei* × garden forms (white filaments), the filaments were coloured, though not so deeply as in *michelmorei*. The green filaments of *dunnii* are unique in *Streptocarpus*. In crosses with garden forms (and with *rexii*), white is completely dominant to green in F_1 and the total numbers from 14 families backcrossed to *dunnii* were 197 white filament : 249 green.

In summary, the results of these studies point to the following conclusions : (1) the inheritance of all the flower patterns is probably monohybrid obscured in some instances by the results of interspecific hybridity, (2) the genes carried by the species are homologous with those found in the garden forms, and (3) genes dominant in the garden forms are usually dominant in the species—a similar situation to that found in the inheritance of the flower colour characters and genes. The species comprising *Streptocarpus* ($2n = 32$) are, therefore, functional diploids.

4. PATTERN IN THE GENUS

The frequency and distribution of seven flower pattern genes and characters are given in table 14. The species are classified on a morphological basis discussed in a paper to follow.

Two major findings emerge from this generic survey. The first relates to the genes *F*, *I*, *B* and *H* and their recessives which are seen to vary in their combinations among the species. In the context of this paper, the three parent species of the garden forms were a random

sample from the genus and all carried *F*, *I*, *B* and *H*, or their recessives, combined as a supergene. Therefore there is no reason to suppose that these genes do not occur combined as a supergene in other species,

TABLE 14

Frequency and distribution of flower pattern genes and characters in Streptocarpus

Taxonomic classification	Gene or character						
Rosulates							
Group I							
rexii	V	F	i	B	H	L	y
„ L	V	F	i	B	H	L	y
„ S	V	f	i	b	H	L	y
„ B	V	f	i	b	H	L	y
„ FB	v	F	i	B	H	L	y
insignis	...	F	*i*	B	*H*	*L*	y
polackii	...	F	*i*	b	*H*	L	*Y*
cyaneus	*V*	F	*i*	b	H	L	Y
gardeni	*V*	F	*i*	b	h	L	y
Group II							
montigena	...	*F*	*i*	b	*H*	*l*	*Y*
meyeri	*V*	F	*i*	b	H	L	Y
johannis	*V*	*F*	*i*	*b*	h	L	*y*
parviflorus	V	f	i	b	H	L	Y
Sub-unifoliates							
Group III							
pusillus	*v*	f	...	...	...	*l*	y
daviesii	...	*F*	*i*	...	...	*l*	*Y*
polyanthus	*v*	F	*i*	b	...	l	Y
gracilis	*V*	*F*	*i*	b	h	l	Y
haygarthii	...	*F*	*i*	b	h	l	y
comptonii	...	*F*	*i*	b	h	l	*Y*
pole-evansii	...	*F*	*i*	b	h	l	*y*
Unifoliates							
Group IV							
grandis	*V*	F	*i*	b?	H	l	y
wendlandii	*v*	*F*	*i*	b	h	l	y
wilmsii	*V*	f	...	...	*h*	L	*Y*
dunnii	*v*?	F	I	b	h	L	y
galpinii	...	*F*	*I*	*b*?	*h*	*l*	*Y*
michelmorei	...	*F*	*I*	b	h	l	Y
eylesii	*V*	*F*	*I*	*b*	...	*l*	...
vandeleuri	*V*	f	...	...	h	*l*	*Y*

Roman type = identified by breeding. Italic type = identified by inspection.

indeed perhaps in all. If they do, then from table 14 it is evident that, in addition to the three combinations characterising *rexii*, *parviflorus* and *dunnii*, viz. *FiBH*, *fibH* and *FIbh*, two others are common, viz. *FibH* and *Fibh*.

The second fact emerging from the survey is that the distribution of the flower pattern genes in general is not random. *I* is found only in the unifoliates ; *B* only in group I rosulates, indeed only in the *rexii* types in this group ; *H*, with one exception, only in the

rosulates. Further, all group I species carry *L* and all group III *l* and it seems that this group may all be *i*, *b*, *h*, *l*. Lastly, the cyanic *rexii* types are all *i*, *B*, *H*, *L*, *y*. Thus species which have been classified into groups on gross morphological criteria are found to be characterised also by their genotypes for flower pattern.

5. DISCUSSION

Origin of garden forms

The origin of the garden forms from artificial crosses made seventy years ago by W. Watson at the Royal Botanic Gardens, Kew, between *rexii* (pale-blue flowers), *parviflorus* (white) and *dunnii* (red) is authentic and the crosses between the parent species and the subsequent development of the garden forms are well recorded and illustrated (*Hortus Veitchii*, 1906). In developing the garden forms, nurserymen selected the rosulate habit of *rexii* (and *parviflorus*) and the redder and/or deeper flower colours made available by the use of *dunnii*. To the eye, the habit, leaves and inflorescences of the garden forms closely resemble *rexii*; therefore the eventual contribution of *dunnii* to flower colour and pattern has been the genes *o R* and *d* determining the various anthocyanin colours (Lawrence and Sturgess, 1957) and the *FIbh* supergene concerned with flower pattern.

These genes from *dunnii* have been incorporated into the *rexii* genome with unmistakable consequences. First, the three parental combinations of the supergene components, *FiBH*, *FIbh* and *fibH*, have remained virtually intact after seventy years of strong selection mainly because mutation of, or crossing-over between, the component units results in gametic or zygotic lethality. Secondly, homozygous garden forms identical with the ancestral species with regard to the supergene, e.g. $\frac{FIbh}{FIbh}$ are no more viable or vigorous, often less so, than other classes, e.g. $\frac{FiBH}{fibH}$. Thus, the *FIbh* supergene is in harmony with the *dunnii* genome but not with that of the garden forms, *i.e.* the *rexii* genome. The incorporation of the supergenes from *dunnii* and *parviflorus* has resulted in a cryptic genotypic complexity which has not been resolved by seventy years of breeding. Indeed the differential zygotic viability and vigour are the overt expression of a hidden genetic unbalance, as also are the deviating ratios. In this last respect there is a close resemblance between the inheritance of flower colour and pattern in the garden *Streptocarpus* and the garden *Verbena*, also of interspecific origin, in which close linkages occur with exceptional frequency (Beale, 1940).

Although the experiments have demonstrated the individual dominance relations of *F*, *I*, *B* and *H* and their recessive alleles, they did not go far enough to establish whether each of the four genes is independent in mutation, though independence of *I* seems clear

(p. 341). Neither was it possible to establish the linear order of the genes on the chromosomes, but the fact that two wild, white-flowered varieties of *rexii*, *rexii S* and *rexii B*, are apparently both *fb* whereas the cyanic species, *rexii*, *rexii L*, also *insignis*, are all *FB*, suggests that these two pairs of alleles are juxtaposed (*cf.* also *parviflorus*). On this point it may be significant that the other wild variety of *rexii*, *rexii FB*, which has white flowers, because it is recessive for the independently inherited gene *V*, is *FB* in constitution.

Flower pattern as an adaptive mechanism

The demonstration that the components of the supergene exist in three different combinations in the three morphologically different species which are the parents of the garden forms, each from distinctly different habitats, suggests that the specific combinations have arisen in response to specific elements of the habitats. Thus, insect pollinators may differ from habitat to habitat. Or the flower pattern of a given species may be the one that is most conspicuous against the particular environmental background, *e.g.* woodland or veldt. In this connection it should be noted that other combinations of the supergene occur in the genus, especially in species from habitats unrelated to those of the ancestors of the garden forms. An alternative view would be that the aggregation of the pattern genes into a supergene was primarily not a direct response to the external environment but an expression of the interdependence of the genes in the control of other essential processes. Whatever the causal mechanism there is little doubt that different species are characterised by a particular combination of co-adapted supergene components.

Taking each of the four genes in turn in the dominant and recessive phases, the total number of possible combinations is sixteen. Two questions are obvious. Do all sixteen occur in the genus ; and what features, if any, are common to species carrying the same combination ? Other patterns may, of course, be controlled by additional components of the supergene, *e.g.* anthocyanin colour in the stigma. Taking the evidence as a whole it seems not unlikely that the supergene constitutes an isolation mechanism.

Flower colour and pattern as the products of evolution

The genetic systems controlling flower colour and flower pattern in *Streptocarpus* are in striking contrast. Flower colour is governed by a number of genes whose inheritance is characteristically independent whereas the genes controlling flower pattern are all linked. In flower colour, gene interaction, denoting synthesis of the pigments from a common precursor or precursors, results in a pigment syndrome in which the different dominant and epistatic genes apparently reinforce one another to maintain high levels of chemical activity and of phenotypic stability. The unifying element, so to speak, is gene interaction. In flower pattern, the unifying element is linkage.

A further difference between colour and pattern relates to wild types. With colour, one type, blue (malvidin 3 : 5-dimonoside) predominates almost exclusively. With pattern, there are a number of wild types comprising, to some extent, groups of species. Thus, colour is widely adapted, pattern much more specifically so.

In this connection, it is of interest that *rexii* and *dunnii* differ with respect to *l*, *B* and *H*, but are alike for *F*, i.e. they differ for highly specific anthocyanin patterns (including intensity) but not for general anthocyanin production.

6. CONCLUSION

Six flower pattern characters studied in the garden forms of *Streptocarpus* are all linked in inheritance, four of them as a supergene in which linkage is complete. The supergene comprises dominant and recessive " alleles " in three combinations only and these are identical with those found in the species known to be the parents from which the garden forms were originally derived by artificial hybridisation. Other species carry other combinations of the supergene and are also characterised, individually and in taxonomic groups, by their flower pattern genes in general.

7. SUMMARY

1. A survey is made of the occurrence of six flower patterns in garden forms and species hybrids in *Streptocarpus*, and of the geographical distribution of these patterns among the species in the wild. Inheritance of the patterns is Mendelian.

2. The gene *F* is necessary for the general production of anthocyanin in the flowers and *I* intensifies the colour. *B* produces a blotch of anthocyanin in the corolla tube and *H* produces anthocyanin in the stalks of the capitate hairs borne on the pistil. *L* controls the production of anthocyanin in lines running down the throat and *Y1*, *Y2* are complementary genes governing a yellow throat stripe.

3. *F*, *I*, *B* and *H* and their recessives are completely linked in specific combinations and comprise a supergene. Eight anomalous forms are shown to have arisen by rare mutation or crossing-over.

4. Only three types of the supergene are found in the garden *Streptocarpus* and these are identical with those of the three species, the hybridisation of which gave rise to the garden forms seventy years ago, viz. *rexii FiBH*, *parviflorus fibH*, and *dunnii FIbh*. Zygotic lethality and sub-lethality result from certain combinations of the components of the supergene.

5. The supergene is linked with *Y2*, and apparently *Y1* is linked with *L*, the cross-over values being 20-30 per cent. Linkage, therefore, may be said to be characteristic of the flower pattern genes in *Streptocarpus*, whereas independent inheritance is characteristic of the flower colour genes.

6. The distribution of the flower pattern genes in the wild is not random, individual species and taxonomic groups being characterised by different combinations of genes and supergene, including those given above.

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A FURTHER ANALYSIS OF VARIABILITY IN *MANIOLA JURTINA* L.

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In a series of papers on *Maniola jurtina*, the Meadow Brown Butterfly, Dowdeswell and Ford (1952-55) have shown that the distribution of spotting on the underside of the hind wings is a sensitive index of micro-evolutionary divergences. The numerical nature of the character scored, the tendency of the insect to form isolated communities, and its overall commonness, all contribute to making it a useful object for evolutionary studies.

1. SPOT-DISTRIBUTION IN THE MALES

Early results established a typical pattern of male spot-distribution with a mode at 2 spots. This pattern was found all over the mainland of England and in the Isles of Scilly. Deviations were found in the Isle of Man, where one population had nearly as many males at 1 spot as at 2 spots, and in Ireland, where two populations were found to show an even greater divergence from the English pattern in that males with 0 and 1 spots were relatively much more frequent. Populations on two islands in the Scilly group, St Agnes' and Great Ganilly, were found to be deviating in the other direction ; that is, having an excess of males at 3 and 4 spots and a deficiency of males at 0 and 1 spots, though in these cases the modes at 2 spots were always just maintained.

It was clear that male spot-distribution could vary, but was being held, presumably by selectional forces, in the familiar unimodal pattern over a large area.

Meanwhile, much more radical variations were found in female spot-distributions. Since these provided the foundation for the present studies of the effects of isolation on micro-evolution, and since time for field-work is often limited by the difficulties of arranging transport between the islands of Scilly, it has frequently been impossible to obtain adequate samples of males. Gradually, however, data on male spot-distributions have accumulated and it is now possible to offer certain generalisations about them.

(1) There is a highly significant correlation between the spot-average in males and the spot-average in females of the same population. Spot-averages are obtained by adding all the spots and dividing by the number of insects. This procedure tends to blur the modalities of spot-distribution in the female but, owing to the prevalence of the

unimodal male distributions, it serves to distinguish those male populations where 0 and 1 spot-frequencies are relatively high from those whose 3, 4 and 5 spot-frequencies are high.

If male and female spot-averages for all years and all locations are plotted (excluding a few collections in which either females are fewer than 20 or males fewer than 25), we find an apparently linear arrangement, with the male mean at 2·11 spots, and maximum and minimum at respectively 2·73 and 1·22 spots, and the female mean at 1·02 spots with maximum and minimum at respectively 1·97 and 0·16 (fig. 1).

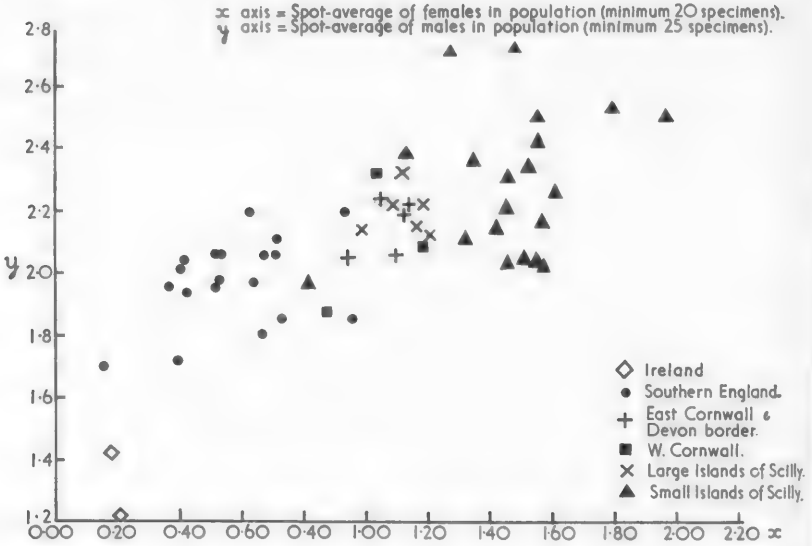

FIG. 1.—Correlation of male and female spot-averages in *Maniola jurtina* 1946-55.

This arrangement of the data makes it quite clear that Ireland (and the Isle of Man) represents an area of low spot-average, and that most of the Scilly group represent an area of high spot-average. The mainland of England is intermediate, though resembling Ireland more than the extreme examples in Scilly. Again, the stabilised group of East Cornwall and West Devon is intermediate between the mainland of England and Scilly. We thus have a general cline in the direction of high spot-average from Ireland, through the Isle of Man, the mainland of Southern England, Cornwall, and the Isles of Scilly. Exceptionally, however, there is one group of populations in Scilly (see below, 2 (v)) which appears to maintain similar spot-averages to those of Southern England but, unfortunately, in most of these cases data of the male distributions are lacking.

On available data, the correlation coefficient may be crudely

estimated at 0·71, which for 54 degrees of freedom is significant at the level of 0·001. (Two entries in fig. 1 happen to coincide.)

The simplest interpretation of this graph and the relationships it indicates is that some or all of the genetic factors which govern the amount of spotting in the insect, and which must be presumed also to influence other characters on which selection is operating, exert parallel influences in both males and females. While the mean of male spotting is consistently higher than that of female spotting, its variance is much less. Variability in the male, in other words, is "geared down". Hence, on first inspection, the male spot-distributions appear much less informative than the female ones. But they are, in fact, also an index of micro-ecological and micro-evolutionary processes.

(2) While female spot-distribution in Southern England has been examined by Dowdeswell and Ford (1953), who obtained samples from several points in the area Rugby-Ipswich-Canterbury-Taunton, and has been shown by them to be extremely homogeneous, at least up to the region of mid-Devon, it is a remarkable fact that male spot-distribution is much less homogeneous.

A study of table 1 illustrates this point. To avoid categories in which expectations are very small, and thus to make a χ^2 test possible, it is necessary to accumulate the values for males at 0 and 1 spots, and at 3, 4 and 5 spots, and those for females at 2, 3, 4 and 5 spots. This gives two 3×13 tables and heterogeneity χ^2 values for 24 degrees of freedom may be computed for the males and for the females of each sample. (One sample collected at Oxford in 1952 has been excluded because it contained only 15 males.) For the females, $\chi^2_{(24)}$ is 32·44; there is thus a probability value between 0·1 and 0·2 that the 13 samples are homogeneous. Considering the varied climates of the places involved and the span of three years over which the samples were collected, this is indeed a sign of massive stability, especially when we reflect that the female spot-distributions are able, under certain conditions of isolation and perhaps other factors, to vary radically in Cornwall and the Isles of Scilly. Turning to the males, however, we find that $\chi^2_{(24)}$ is here 52·68, showing that this is a highly heterogeneous collection of samples, and that such variety could only be expected by chance in fewer than 1 in a 1000 of such collections if they were from a homogeneous population. When we search these readings of male insects for possible causes of this variation, we find that there is a strong suggestion of an east-west cline. This is discussed under section (3) below.

The very few populations studied in Ireland and the Isle of Man suggest that an extreme of male variability may occur in this region, coupled with the general cline in the direction of spot-reduction. Again, in the South-West, in those isolated populations in the Scilly group where the trend is towards high spot-averages, the males may also be more variable than in Southern England. Nowhere, however,

within the limits of our available data, do the male spot-distributions achieve the extraordinary homogeneity of the females in Southern England—a homogeneity which, as Dowdeswell and Ford (1952, 1953) point out, overrides very great differences in ecology.

(3) There are strong indications that a subsidiary cline exists in the males of the highly stabilised populations of Southern England. Readings from Lundy Island (off the North Devon coast) and from Oxford, Rugby and Taunton, suggest that these western populations, though conforming to the general picture of stabilisation, tend to have low spot-averages, while the readings from Canterbury and

TABLE 1

Spot-distributions of South England samples 1950 to 1952, excluding Devon and Cornwall

Spot numbers	Male						Total	Female						Total
	0	1	2	3	4	5		0	1	2	3	4	5	
Rugby 1950 .	3	8	31	7	...	...	49	26	10	6	1	[illegible]	...	45
Canterbury 1950 .	1	4	32	11	1	1	50	24	11	10	4	1	...	50
Ipswich 1950 .	3	15	61	20	2	1	[illegible]	63	21	17	4	...	1	106
Taunton 1950 .	0	3	45	4	1	...	53	18	6	3	1	...	...	[illegible]
Salisbury 1950 .	1	6	39	13	2	1	62	26	8	8	1	...	...	43
Oxford 1950 .	1	7	36	4	1	...	[illegible]	37	12	4	1	...	...	54
Ipswich 1951 .	1	6	41	7	1	1	57	70	22	9	5	...	...	106
Salisbury 1951 .	5	11	32	5	...	...	53	22	2	1	...	...	...	25
Oxford 1951 .	1	3	40	3	...	...	47	30	10	3	...	...	...	43
Ipswich 1952 .	4	9	73	13	1	...	100	61	22	16	4	...	...	103
Salisbury 1952 .	2	6	55	9	1	..	73	41	[illegible]	6	1	...	...	56
Winchester 1952 .	8	26	160	26	4	1	225	77	25	12	3	1	...	118
Taunton 1952 .	8	23	90	8	3	...	132	21	15	7	...	...	...	43
Total . .	38	127	735	130	17	[illegible]	1052	516	172	102	25	4	1	[illegible]

Ipswich suggest a somewhat higher spot-average. Within the stabilised area, however, these differences are very slight and have not been very constant from year to year.

If such a cline in the male spot-distribution exists, we should expect to find that samples from each of the two sides of the whole area of Southern England, *i.e.* a western group and an eastern group, would be more homogeneous than both the groups taken together. We have seen in (2) above that the males across the whole of Southern England are in fact highly heterogeneous ; but if we select the five samples obtained at Rugby, Oxford and Taunton, we find that $\chi'_{(8)}$ is only 14·90 ; P then lies between 0·05 and 0·1. This indicates some approach towards homogeneity. It will be observed, however, from table 1 that there is considerable variation between years in the two pairs of samples from Taunton and Oxford. If we take the further step of accumulating the samples from Taunton and the samples from Oxford, adding also a sample taken at Oxford in 1952, which contained only fifteen males and which was, therefore, too small to stand on

its own in a heterogeneity calculation, we find that $\chi^2_{(4)} = 4{\cdot}36$ and P lies between 0·3 and 0·5. (The Oxford sample of 1952 contained two males at 0 spot, two at 1 spot and eleven at 2 spots.) This indicates a very considerable degree of homogeneity.

Similarly, if we turn to the eastern wing of the Southern England populations and compare the samples taken at Canterbury and Ipswich, it is found that $\chi^2_{(6)} = 6{\cdot}91$, and that P lies between 0·3 and 0·5, again indicating considerable homogeneity. No substantial difference is made by accumulating the three Ipswich samples taken in the years 1950 to 1952, since $\chi^2_{(2)} = 2{\cdot}22$, and the value for P is hardly changed.

The question also arises whether a population sampled on Lundy Island in 1955 may legitimately be included with the Southern England collections, as suggested by its female spot-distribution. Forty-six males were taken and of these five had 0 spots, five had 1 spot, thirty-four had 2 spots and two had 3 spots. This sample seems to be tending in the direction of the Irish populations. Lundy lies in the Bristol Channel, twelves miles off the North Devon coast ; its population of *M. jurtina* will, of course, be completely isolated from those of the English mainland. If the Lundy sample is included in a heterogeneity calculation with the populations of Rugby, Taunton and Oxford, $\chi^2_{(10)} = 16{\cdot}32$, and P lies between 0·05 and 0·1. If we aggregate the samples taken in different years at the same place and include the Lundy sample, $\chi^2_{(6)} = 5{\cdot}86$ and P lies between 0·3 and 0·5. Thus we find that the inclusion of Lundy does not affect the homogeneity of the western group of South England samples. This is the first indication that the stabilisation found in South England may result from independent selectional adjustment of isolated populations as well as from gene-flow across the whole area.

The data of table 1 do not indicate any regularity in the variations from year to year which are so characteristic of the male samples from each location. Together with the west-east cline, however, they suffice to produce a heterogeneous male population alongside a strictly homogeneous female population. Nevertheless, there remains the general correlation between male and female spot-averages ; it seems that within the area of stabilisation, male spotting reacts to some micro-evolutionary influence more readily than female spotting. Thus, while *jurtina* conforms to the rule of greater female variability over its whole range (Fisher and Ford, 1928), there can be a tendency towards greater male variability within the various stabilisation areas.

Clearly more extensive data are needed, not only from Southern England but also from its periphery, *i.e.* Wales, Northern England, Northern France and the Isle of Wight. If further data from Southern England establish this suggested cline, we should have a picture of extreme low spot-average in Ireland, and extreme high spot-average (*a*) in the Scilly group and (*b*) in East or South-East England. The cline in the direction of South-East England would be " damped ",

possibly through lack of isolation, and would express itself much more markedly in the males, while the cline in the direction of Scilly would be "damped" only as far as the critical Devon-Cornwall boundary, west of which isolation, and perhaps other ecological factors, permit wide variations which are here, however, chiefly expressed in the females.

2. FEMALE SPOT-DISTRIBUTIONS IN THE ISLES OF SCILLY

It is now becoming possible to discern certain patterns in the female spot-distributions.

A summary of the various types of female populations so far found might be useful.

(i) *The large island group*

On St Mary's, Tresco and St Martin's, the females are independently stabilised, showing roughly equal frequencies at 0, 1 and 2 spots; each provides about 30 per cent. of the total with a "tail" of about 10 per cent., where the spots are 3, 4 or 5. The effect of plotting the distribution of these populations is to give a "flat-topped" curve. This stabilisation has been shown to be very strong, both from year to year and from island to island. Dowdeswell and Ford (1955) have attributed this to the need for a population covering an area of mixed ecology to achieve a compromise (and to equilibrate gene-frequencies).

(ii) *Unimodal populations with a mode at 2*

These have been found on St Helen's, St Agnes', Samson South and Bryher (Gweal Hill). This last population and the population on St Helen's (in 1953, but not in 1951) gave large readings for 0-spot and 1-spot. They thus tended to approach the "flat-topped" distribution of the large islands.

(iii) *Bimodal populations with a large mode at 2 and a lesser mode at 0*

These have been found on Tean One, Tean Three, Arthur, Tresco Farm (in 1955), Samson North, and Old Man North.

(iv) *Bimodal populations with a large mode at 0 and a lesser mode at 2*

These have been found on St Martin's South-East and St Martin's North-West (two isolated areas of one of the "large" islands), and on Old Man South (though here with a high "tail").

(v) *Unimodal populations with a large mode at 0*

These have been found on Bryher South, White Island, and on St Martin's opposite White Island.

(vi) *Two unstable populations*

The population on Tean Five and that on Great Ganilly have moved between (ii), (iii) & (iv) and (iii), & (iv) respectively.

It will be seen that when spot-average is considered, (ii) and (iii) will tend to fall into a high spot-average group, and (iv) and (v) into a low spot-average group. (i) will be intermediate.

It is possible to plot these female spot-distributions in such a way as to make any " clumping " of separate populations detectable. The frequencies of 0, 1 and 2 spots are expressed as a percentage in each population (the " tail " being for the moment disregarded). The three values are then plotted on a triangular graph (fig. 2 ; and Appendix). When this is done, four main points are easily observed :

(*a*) The " small island " populations are divided sharply into two

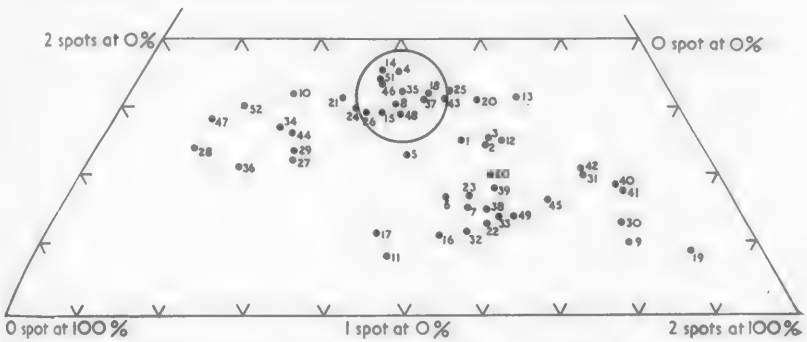

FIG. 2.—*Maniola jurtina* : Isles of Scilly—females 1946-1955. Within all populations the number of specimens at 1 spot (expressed as a percentage of the total at no spot, 1 spot and 2 spots) never exceeds 35 per cent. All large island populations fall within the circle.

parts : (i) those with 2 spots below the 30 per cent. level, and (ii) those with 2 spots above the 39 per cent. level. There are *no* " small island " populations with 2 spots between 30 and 39 per cent.

(*b*) Into the gap fall the highly homogeneous populations of the large islands, which *all* have 2 spots between the 30 and 40 per cent. levels.

(*c*) No population has " one spot " in excess of 35 per cent., though very many approach this figure. (If mainland populations are added, this is even clearer : there is one exceptional and, on other grounds, inadmissible, population (Hayle, 1951) with 1 spot at 45 per cent. It is not certain that this sample was collected from one locality ; the 1952 sample (Dowdeswell and Ford, 1953) was quite different). It appears that some populations are " pressed " against the 1 = 35 per cent. line. There must be some factor in the genetic control of spot-numbers which prevents the frequency of 1-spot exceeding 35 per cent. of the total of the 0-spot, 1-spot and

2-spot phenotypes. Nothing is, as yet, known about the individual genetics of spot-numbers since the insects have not been bred under laboratory conditions owing to the difficulty of mating them.

(*d*) " Tail " percentages (*i.e.* the proportion of 3-spot, 4-spot and 5-spot specimens) vary more or less according to the 2-spot level.

It is clear that the female spot-distributions of the various populations in Scilly are not randomly scattered. Within the limitation that 1-spot may not exceed 35 per cent., there is a clear tendency to " clump " into three groups, with a possibility of a fourth.

It is a reasonable first approximation to suggest that these groups reflect independent adjustments to at least three different types of ecology, possibly governed by the flora :

(*a*) Mixed—where, as on the large islands, a population has to compromise between various ecologies, and adopts the " flat-topped " distribution.

(*b*) Open—in the enclaves of St Martin's and on White Island (Old Man South, with a very high " tail ", may not really belong to this group). Here the trend is towards low spot-average and a mode at 0.

(*c*) Luxuriant—in most, but not all, of the other islands where there is a high spot-average and the female distributions are unimodal at 2 or bimodal with a high mode at 2.

This suggestion that the types of flora influence the specialisation of *jurtina* into high-spot and low-spot groups is necessarily very tentative, and will, of course, need confirmation from future observations. Exceptionally, the two isolated populations on Bryher do not conform to this pattern. The Gweal Hill habitat is more exposed than that of the southern part of the island, so that one would expect the spot-distribution patterns to be reversed. These two populations are, however, the only noticeable exceptions so far recorded to a possible generalisation that the spot-distributions tend to be adjusted to different types of ecology.

3. SYNOPSIS OF AREAS

Taking each area of the British Isles, on which data are available, in turn, one finds the following position :

(i) *Ireland and Isle of Man*

Lowest spot-averages in both sexes (♂ about 1·3 ; ♀ about 0·2).

Probable high variability in males, tending to destroy the mode at 2.

Probable high stability in females ; very large mode at 0.

The only Isle of Man distribution so far obtained is intermediate between those of Ireland and those of South England (Dowdeswell and Ford, 1953).

(ii) *South England*

Low spot-averages in both sexes (♂ about 2·0 ; ♀ about 0·6).
Moderate variability in males, but not disturbing the mode at 2.
Extremely high stability in females with a large mode at 0.

(iii) *East Cornwall and Devon-Cornwall border*

There is an abrupt change in the course of a few miles (Dowdeswell and Ford, 1953) to a series of populations which are stabilised on a different basis. These are characterised by medium spot-averages in both sexes (♂ about 2·2 ; ♀ about 1·1).

Probable high stability in males with mode at 2.

High stability in females with major mode at 0, and minor mode at 2. Dowdeswell and Ford (1953) have shown that five samples of females from an area measuring 25 × 20 miles are homogeneous.

(iv) *West Cornwall*

M. jurtina appears to be much divided into isolated groups here so that the picture is rather similar to that of the Isles of Scilly, except that no " flat-topped " distributions have been found. Spot-averages are medium in both sexes (♂ about 2·1 ; ♀ about 1·0).

Not very many data on the stability of males are available, but the females show considerable variation.

(v) *Isles of Scilly*

There is a wide range of spot-averages here, though the general tendency is towards high counts ; the highest counts ever recorded are in small islands of the group.

The spot-averages of the highly stabilised " large island " group are similar to those of East Cornwall (♂ about 2·2 ; ♀ about 1·1), but the " flat-topped " female distribution with 0, 1 and 2 spots about equal, each at 30 per cent. of the whole, is characteristic of the group and found nowhere else.

Where the same isolated populations have been sampled in different years, they are usually highly stable, but extremely rapid shifts have been observed in Tean Five, where they were clearly associated with sudden ecological changes (Dowdeswell and Ford, 1955). The population on Great Ganilly has also changed between 1951 and 1955, but not so rapidly (Dowdeswell, Ford and McWhirter, 1957). Here no obvious change in the ecology has been detected.

4. DISCUSSION

Many cases of sex-controlled polymorphisms are known in Lepidoptera ; examples of these are given by Ford (1945). It is clear from this analysis of the spot-number variations of *M. jurtina* that the insect is equipped to adjust the stability of the phenotype ratios independently, or in both sexes. Yet while either sex may

be stable or unstable, the overall correlation between the spot-averages in males and females always remains high. As the picture obtained of the spot-distributions in *M. jurtina* has expanded, so the extent of the insect's potential variability has become clearer. Three groups of populations showing highly stabilised female distributions and extending over habitats of varied ecologies have been found in Southern England, East Cornwall and the "large island" group of Scilly; each differs markedly from the others. Of these, at least the Scilly group is composed of rigorously isolated populations. All three groups must be presumed to retain the ability to vary their phenotype ratios, when selectional forces require it, but, except in the case of South English males, they have not been found doing so. In small populations, under the influence of isolation and consequent restriction of the variation in the ecology of the habitat, certain adjustments seem regularly to develop, so that the spot-frequencies are found to be altered.

A point of major theoretical interest arises when one asks the questions "Is variability in these isolated groups *latent, or altogether lost*?" and "Are modifying genes being worked out of the isolated populations, or does selection for success in a specialised habitat merely depress the level of some of them?" Probably no population small enough to be affected by "genetic drift" has yet been studied. The indications, however, are that the consistent distributions arrived at independently by isolated populations, especially on Scilly, are due to selective influences. The rapid response of the population of Tean Five to observed ecological changes (Dowdeswell and Ford, 1955) strongly suggests that selection can press an isolated population into a "corner" from which it will rebound when conditions are altered. There is no evidence that any modifiers have as yet been eliminated.

There is a striking parallel between the behaviour of *M. jurtina* with respect to spot-distribution in the British Isles and that of peripherally isolated populations in general, of which the *Tanysiptera hydrocharis-galatea* group of kingfishers in New Guinea, described by Mayr (1954), is an interesting example. In the case of the kingfishers differentiation has gone as far as speciation, while even subspeciation is rather doubtful in *M. jurtina*. Mayr finds that the central habitat of *T. galatea*, though covering enormous variations in climate, flora and fauna, contains a highly stabilised population, while the peripheral and isolated islands usually contain species which are highly differentiated, even from mainland species occupying areas of similar climatic conditions—a situation which has been described in many other animal species.

Mayr considers that both the "selectional" and "drift" theories are inadequate to explain the observed variations in *Tanysiptera*. He suggests that the chief influence on peripherally isolated populations is the revaluation of the selective advantages of genes in the new genetic environment that results from sudden isolation and intensive

inbreeding of the " founder " genotypes. This precipitates the building up of a new gene complex in the isolated population.

It may be that *M. jurtina* is at the beginning of an evolutionary process which could lead to the condition described by Mayr in *Tanysiptera*. If variation in *M. jurtina* is an incipient micro-evolutionary process, we should expect external factors, *e.g.* selection based on ecological differences, to predominate ; probably all but the very smallest groups of " founders " would contain all the alleles influencing spotting. In this way any colony likely to survive would adjust itself to its ecology after a few generations by using the same genetic potential. It is possible, however, to visualise a more advanced stage in such evolution after which sufficient incompatibility of genotypes would have accumulated to make the composition of a " founder " population of prime importance. At this stage, certain further outside genes accruing to the new population would not simply be homogenised, causing a momentary disturbance of the gene-equilibrium ; they would instead be confronted with a new genetic stabilisation and would be absorbed only rarely—subspeciation would then have begun.

At the present time, however, it seems likely that all populations of *M. jurtina* contain sufficient variability in respect of factors governing spot-numbers to establish whatever distribution is most advantageous, and that all these adjustments are being carried out against the same genetic background. The sudden change in Tean Five shows that all the genetic material required for coping with the new ecological situation was instantly available for selection, in a case where the " founder " principle is inapplicable. Owing to the characteristics of *M. jurtina* and its habitats in Scilly, it is reasonable to hope that direct experimental tests will provide answers to some of these problems.

A further parallel between Mayr's observations and the present analysis is that the cline described in the *M. jurtina* males of South England occurs in a population whose females are highly stabilised. The populations sampled in South England are likely to be imperfectly isolated. Gene-flow is thus preventing even the beginnings of subspeciation. The continual operation of selection can do no more than produce a slight, but significant, cline in the males, which in its turn is but dimly reflected in the stable females. Given isolation, however, as in West Cornwall and in the Isles of Scilly, the potential variability can be utilised and the first, though probably reversible, steps in the direction of subspeciation can be taken.

5. SUMMARY

1. A highly significant degree of correlation has been found between the average spot-number in males and in females of *Maniola jurtina* all over the British Isles.

2. Though male spot-distributions generally vary less than females and always maintain a mode at 2 spots, they are highly heterogeneous

across Southern England in those places where females have been shown to be highly homogeneous.

3. Apparently the spot-distributions in each sex can be adjusted so that one sex may be comparatively labile and exhibit clines, while the other is highly stabilised.

4. The existence of a cline in both sexes from low spot-averages in Ireland, through the Isle of Man, Southern England, Cornwall, to high spot-averages on the Scilly group is established. A probable " damped " cline from low spot-average in western South England to a higher spot-average in eastern South England which is chiefly reflected in the males, is discussed.

5. It is established that female spot-distributions in isolated populations of the Scilly group tend to " clump " at certain patterns. They must have arrived at these independently under the influence of selectional forces.

6. A tentative identification of variations in the flora with the operative selectional force determining the pattern of female spotting is made.

7. *M. jurtina* behaves at its periphery as many animal species do, but the isolated populations do not appear to have as yet evolved inter-genotype incompatibility, at least so far as the factors governing spot-number are concerned. Experimental work on *M. jurtina* could provide further direct tests of the validity of various genetic theories of evolution.

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APPENDIX

Data from which fig. 2 is compiled

Ref. no.	Place	Year	Percentages of total at (0+1+2 spots)		
			0 spot	*1 spot*	*2 spots*
1.	Tean One	1946	30	25	45
2.	Tean Three	1946	27	24	48
3.	Tean Five	1946	26	25	48
4.	*St Martin's*	*1950*	33	35	32
5.	Tean One	1950	38	23	39
6.	Tean Three	1950	36	17	47
7.	Tean Five	1950	34	16	50
8.	*Tresco*	*1950*	36	31	34
9.	Arthur	1951	16	10	73
10.	Bryher	1951	48	32	20
11.	Great Ganilly	1951	48	8	44
12.	St Agnes'	1951	25	25	50
13.	St Helen's	1951	20	31	49
14.	*St Martin's*	*1951*	35	35	30
15.	*St Mary's*	*1951*	38	29	33
16.	Tean One	1951	40	11	49
17.	Tean Five	1951	48	12	41
18.	*Tresco*	*1951*	31	32	37
19.	Arthur	1953	9	9	82
20.	Bryher (Gweal Hill)	1953	25	31	44
21.	Bryher (South)	1953	42	31	27
22.	Great Ganilly	1953	33	13	54
23.	Old Man North	1953	33	17	50
24.	Old Man South	1953	41	30	30
25.	St Helen's	1953	28	32	40
26.	*St Martin's*	*1953*	40	29	31
27.	St Martin's (NW.)	1953	53	22	25
28.	St Martin's (opp. White Island)	1953	64	24	12
29.	St Martin's (SE.)	1953	52	23	25
30.	Samson North	1953	16	13	71
31.	Samson South	1953	17	20	63
32.	Tean One	1953	36	12	52
33.	Tean Five	1953	31	14	55
34.	White Island	1953	52	27	21
35.	*St Martin's*	*1954*	34	32	34
36.	St Martin's (opp. White Island)	1954	60	21	19
37.	*St Mary's*	*1954*	31	31	37
38.	Tean One	1954	32	15	53
39.	Tean Three	1954	30	18	52
40.	Tean Four	1954	14	18	67
41.	Tean Five	1954	13	18	69
42.	Tresco Farm	1954	17	21	62
43.	*Tresco*	*1954*	29	31	40
44.	White Island	1954	51	26	23
45.	Great Ganilly	1955	24	16	60
46.	*St Martin's*	*1955*	36	33	31
47.	St Martin's (opp. White Island)	1955	60	28	12
48.	*St Mary's*	*1955*	36	28	35
49.	Tean One	1955	29	14	57
50.	Tresco Farm	1955	29	20	51
51.	*Tresco*	*1955*	36	34	30
52.	White Island	1955	55	30	15

Places italicised are the main areas of the "large island group"; these are all independently stabilised at the "flat-topped" distribution. All these samples, and these samples only, fall within the circle in fig. 2.

THE ORGANISATION OF POLYGENIC ACTIVITY WITHIN A CHROMOSOME IN *DROSOPHILA*

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THE response of a population of living organisms to the impact of any force of selection will vary not only with the nature of the force itself but also with the genetical architecture of the character or characters in question. The genetical architecture of a character will of course reflect those general properties which together constitute the genetic system of the population and species (Darlington, 1939, 1956) : the system of reproduction, whether asexual, subsexual, sexually inbreeding or sexually outbreeding ; and the number, organisation and relations of the chromosomes especially as reflected in the properties of genic recombination. At the same time, the architecture will depend on the detailed organisation (both mechanically in hereditary transmission, and physiologically in developmental action) of the system of genes mediating its expression, or rather the variation in that expression. Furthermore, in considering the organisation of the genic system, and particularly the polygenic system, mediating the variation in one character we cannot neglect its relation to the genic systems affecting other characters, for selective response in the one will be conditioned by any effect it may have in dragging other characters with it, whether because of the linkage relations of the two gene systems along the chromosomes or of their physiological relations in action. All these things must be taken into account if we are to understand response to selection ; and each will tell us something of the past action of selection, for the systems and organisations we see to-day will be those which have successfully survived the test of selection in the past.

At present we have little detailed knowledge of the organisation and properties of polygenic systems in these respects. Selection experiments have told us something of their overall properties in a number of species, particularly *Drosophila* and mice ; and, in *Drosophila*, chromosome assays and similar techniques have shown us broadly how the polygenic system is characteristically distributed over all the chromosomes, how its member genes in different chromosomes can interact in producing their effects, and how interactions can be seized on by selection. About the distribution within chromosomes and the relation of interactions to that distribution, however, little is known. Early analysis (Mather, 1942, and see review by Wigan, 1949*a*) did little more than show that the problem existed, though

in his own experiments Wigan was able to establish the occurrence of polygenic activity along the whole length of the X chromosome of *Drosophila*. More detailed and more precise information is, however, needed about both gene distribution and gene interaction if we are to understand the consequences of selection or to obtain the insight into gene structure and function that such studies offer (Mather, 1954). The present paper and its companions report an investigation aimed at supplying information of this kind.

1. CONSTRUCTION AND USE OF THE RECOMBINANT CHROMOSOMES

Investigation of the distribution of polygenic activity along a chromosome requires the use of marker genes to enable particular segments of the chromosome to be identified and followed in the breeding experiments. In past experiments these marker genes have been used directly to identify the presence of recombinants whose effects on the expression of the primary character could then be assessed immediately without further breeding. This direct approach is convenient and relatively quick but it suffers from certain drawbacks, notably that the marker genes themselves sometimes disturb the character in question so that assessment of the effect of the segments of chromosome they mark becomes difficult, and that it is impossible to investigate fully the interactions in effect of the different segments. A different technique was therefore used permitting the construction, storage and subsequent use of recombinant chromosomes from which all marker genes had been eliminated.

The investigation was begun from two stocks, one selected for high and the other for low number of abdominal chaetæ. The former, which will be denoted as H, was descended by mass mating from the 8×9 line of Mather and Harrison (1949), and the latter, which will be denoted as L, was descended from the LL line of Harrison (1953). The average chaeta numbers of the two were found to be H $61{\cdot}46 \pm 0{\cdot}45$ and L $36{\cdot}19 \pm 0{\cdot}14$. A small chromosome assay of the customary type traced a difference of 4·80 chaetæ to genes in the X chromosome, one of 3·35 chaetæ to genes in chromosome II and one of 8·20 chaetæ to chromosome III, a total of 16·35 chaetæ where on an average no more than half the parental difference is expected to be assignable to chromosomes by this type of assay. Attention was therefore concentrated on chromosome III as offering the largest genetical difference for analysis.

Both H and L were crossed to a stock carrying the so-called "rucuca" chromosome III, which bears the genes *ru*, *h*, *th*, *st*, *cu*, *sr*, e^s, *ca* marking its whole length (see fig. 1). The genes *th* and *st* are, however, so close together on the genetical map that it was found preferable to treat them as a single composite locus, the rare recombinations between them being discarded. This composite locus is referred throughout as *st*, and the average limits of the origins shown

in fig. 1 have been determined from the position of *st*. This will not disturb the right limits of regions B, P and X but will mean that the left limits of these regions are shown just under half a unit too far to the right—a difference which must be small when compared with the intrinsic variation in the position of the effective point of crossing-over. Heterozygous females of both types, $\frac{\text{H}}{\text{rucuca}}$ and $\frac{\text{L}}{\text{rucuca}}$, were backcrossed to rucuca males, so that recombinant chromosomes consisting partly of the H (or L) chromosome III and partly of the rucuca chromosome III could be recognised. Thereafter the procedure varied in detail with the particular type of recombinant chromosome aimed at, but in general it was the same for all and it will be illustrated

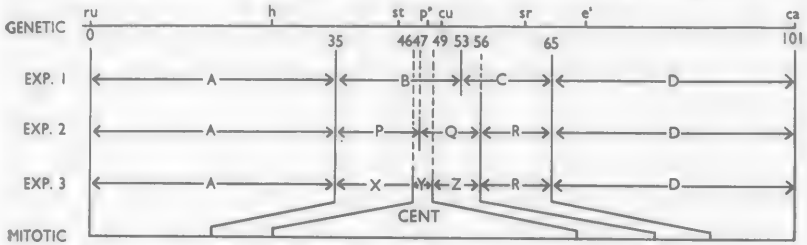

FIG. 1.—The genes used in constructing the wild-type chromosomes and the segments of which, in consequence, these chromosomes were constructed (see in the text).

The extent of each segment is indicated by the arrows, the limits being shown in their average positions. Apart, however, from the left of A and the right of D, the limits might fall by the vagaries of recombination anywhere between the straddling genes (*e.g.* the right limit of A might come anywhere between *h* and *st*) the average position being midway between them on the genetical map. This variation in position of recombination could also lead to a small piece of the marked chromosome being inserted in the region of the limit.

The average position of all the limits are indicated in relation to the genetical map (above) and of some of them also in relation to the mitotic cytological map (below). The general position of the centromere (Cent.) is shown on the mitotic map, though it may well be nearer to the left limit of region Y than is indicated. All positions on this map are very approximate.

by reference to the construction of chromosomes showing recombination between *h* and *st*. From the progeny of backcrossing $\frac{\text{H}}{\text{rucuca}}$ to rucuca a fly of the phenotype *st cu sr* e^s *ca* and therefore of genotype $\frac{\text{A } st\ cu\ sr\ e^s\ ca}{ru\ h\ st\ cu\ sr\ e^s\ ca}$ (where A indicates a segment from the H chromosome III covering the *ru* and *h* loci) was selected and mated to a fly from the $\frac{\text{L}}{\text{rucuca}}$ backcross of the opposite sex and of phenotype *ru h* and therefore of genotype $\frac{ru\ h\ \text{b}\ \text{c}\ \text{d}}{ru\ h\ st\ cu\ sr\ e^s\ ca}$, where b c d represent a segment of the L chromosome III covering the *st cu* e^s *ca* loci. Wild-type daughters which must be of the constitution $\frac{\text{A } st\ cu\ sr\ e^s\ ca}{ru\ h\ \text{b}\ \text{c}\ \text{d}}$ were

taken and crossed again to rucuca males. The wild-type progeny of this cross must bear a chromosome combining a segment of H at the left end and a segment of L at the right end, the junction lying between *h* and *st* with possibly a short piece of chromosome from rucuca interposed between H and L segments. A male of this type was then mated to a stock having its two chromosomes III marked by *Me Sb* and *H* (= gene Hairless) respectively. The recombinant chromosome can then be held in the male line, sheltered from further recombination, by crossing *Me Sb*/*H* females to *Me Sb*/recombinant males in every generation. The desired wild-type chromosome,

TABLE 1

The recombinant wild-type chromosomes constructed and used in the experiments. Capital and small letters indicate segments derived from chromosome III of the H and L lines respectively. The limits of the segments denoted by A-a, B-b, etc. are shown in fig. 1. In Group (c) the constitution in respect of segment D-d is not fully certain as the gene ca *was omitted from the marked chromosome used in the process of construction.*

Group	(*a*)	(*b*)	(*c*)	
Parental chromosomes	(1) ABCD = APQRD = AXYZRD (2) abcd = apqrd = axyzrd			
Recombinant chromosomes	(3) aBCD (4) abCD (5) abcD (6) ABCd (7) ABcd (8) Abcd	(9) APqrd (10) APQrd (11) apQRD (12) apqRD	(13) AxyZR(D) (14) AxYZR(D) (15) AXYzR(D) (16) AXyzR(D) (17) AxyzR(D)	(18) axyZr(d) (19) axYZr(d) (20) aXYZr(d) (21) aXYzr(d) (22) aXyzr(d)

composed of known pieces from the H and L chromosome III, has been constructed and put into store.

Recombinant chromosomes were constructed in three separate rounds. In the first round a modified rucuca chromosome was used from which *cu* was omitted for reasons which need not detain us. No recombinants were selected in the end segments between *ru* and *h* or e^s and *ca*, so that the chromosome was being treated in effect as consisting of the four segments A, B, C and D as shown in fig. 1. The end points of the segments are of course not sharply defined since the right end of segment A, for example, may come anywhere between *h* and *st* and need not in fact coincide exactly with the left end of segment B. Thus chromosomes which are grossly similar in terms of the segments they have derived from H and L, need not agree precisely in the points at which recombination has fixed the ends. The average position of the end will be the mid point of the two marker genes on the genetical map. The recombinant chromosomes of table 1 (*a*) were constructed in this way, two separate and distinct chromosomes of each type being made up on this and all later occasions. Capital letters indicate segments from the H stock and small letters the corresponding segments from L.

In the second round a usable rucuca chromosome was available carrying *cu*. The use of *cu* as a marker between *st* and *sr* enables the two segments, B and C of the previous occasion to be broken up into three, denoted as P, Q and R in the way shown in fig. 1. Four types of chromosome were built up as shown in table 1 (*b*).

A further gene, p^p, was introduced into the marked chromosome for the third round of chromosome building, the gene *ca* being left out so as to avoid difficulties of classifying the flies. The introduction of p^p enables the segments P and Q to be jointly broken down into three segments, denoted by X, Y and Z (fig. 1). Ten types of chromosome were constructed as shown in table 1 (*c*). Since *ca* was omitted, the status of the chromosomes was not fully clear in respect of part of segment D.

The various recombinant chromosomes were tested for their effects on the various characters chiefly in sets of diallel crosses, as will be detailed later. In respect of chromosome III the crosses used in these diallels were always of the type $\frac{\text{I}}{Me\ Sb} \times \frac{\text{J}}{H}$ where I and J indicate any two of the recombinant chromosomes. The *Me Sb* chromosome materially reduces, if it does not wholly suppress, recombination in the female parent, so preserving the identity of chromosome I. Such a cross gives four types of progeny $\frac{Me\ Sb}{H}$; $\frac{\text{J}}{Me\ Sb}$; $\frac{\text{I}}{H}$; $\frac{\text{I}}{\text{J}}$. The last class of flies, which is of course wild type, provides the material for assessing the joint effect of the I and J chromosome on the various characters such as chaeta number. The first type appears in all crosses and therefore affords a yardstick for the measurement of the effects of the recombinant chromosomes on the viability of their carriers.

The background of X and II chromosomes was held constant or virtually so within each diallel set, coming either from the $\frac{Me\ Sb}{H}$ stock (and denoted as the M background) or the Oregon stock (and denoted as the O background). The recombinant chromosomes were placed on the M background by backcrossing into the marked stock. Both these and the marked chromosomes were placed on the O background in the same way. At least four generations of backcrossing were used in every case.

2. THE FIRST EXPERIMENT

This was based on a set of diallel crosses including all the chromosomes (1)-(8), with the exception of (2), as listed in table 1. Every chromosome was introduced from the mother into combination with every chromosome from the father. With 7 chromosomes there are thus 49 combinations of which 7 are homozygotes. The 42 heterozygous combinations fall into 21 pairs, the members of a pair differing

according to which of the two chromosomes came in from mother and father respectively. The differences between the members of these pairs afford information about differences between reciprocal crosses, and provided such reciprocal differences are small the members of a pair can be pooled for comparison with the other combinations, both heterozygous and homozygous.

The diallel set was made up four times on separate occasions, twice with one group of recombinant chromosomes and twice with a second group formally like the first in respect of segments A-a, B-b, C-c and D-d, but constructed separately so that they may differ from the first group as a result of the normal variation in the position of crossing-over between the marker genes. Two diallel sets were made with the M and two with the O backgrounds, backgrounds and groups of chromosomes being combined in all ways, thus, M background-1st group, M background-2nd group, O background-1st group, O background-2nd group. The numbers of chaetæ on the 4th and 5th abdominal segments, and the numbers of sternopleural chaetæ on both sides of the fly were counted on 10 wild-type females and on 10 wild-type males of each combination. In 14 cultures fewer than the normal 10 flies of each sex were obtained and the reduced numbers of flies had to be accepted as the basis for evaluation in these cases. The combination (4) (3) failed completely in the third diallel set. The relevant mean numbers of chaetæ were then estimated by the missing-plot technique based on reciprocal differences. All the calculations to be described were made using as data the mean number of abdominal chaetæ on the 4th and 5th segments together, or the mean number of sternopleural chaetæ on the two sides together, from the 10 females and 10 males of each combination.

A preliminary analysis of each diallel set was made by the calculation and comparison of the array variances (V) and covariances within arrays of family mean and non-recurrent parent (W) described by Hayman (1954*b*) and Jinks (1954). The W/V graphs calculated from the overall means of the combinations, obtained by averaging each combination over sexes, backgrounds and groups (fig. 2), showed no significant departure from a straight regression line of unit slope, though the points did suggest a slight curvature, which is fairly attributable to the partial association of the different alternative segments in the parental recombinant chromosomes. A further graphical comparison was made between the covariance (W) described above and a further covariance (W^1), similar to it and again calculated within the array, but relating the family mean not to the non-recurrent parent but to the mean of all offspring of that parent (Hayman, unpublished). Interaction reveals itself in such a graph (fig. 2) by departure of the regression of W on W^1 from a line of slope 2. In the present case the slope falls a little below this expectation, but the discrepancy may reasonably be attributed to inequality of the frequencies with which alternative segments, such as A and a appear

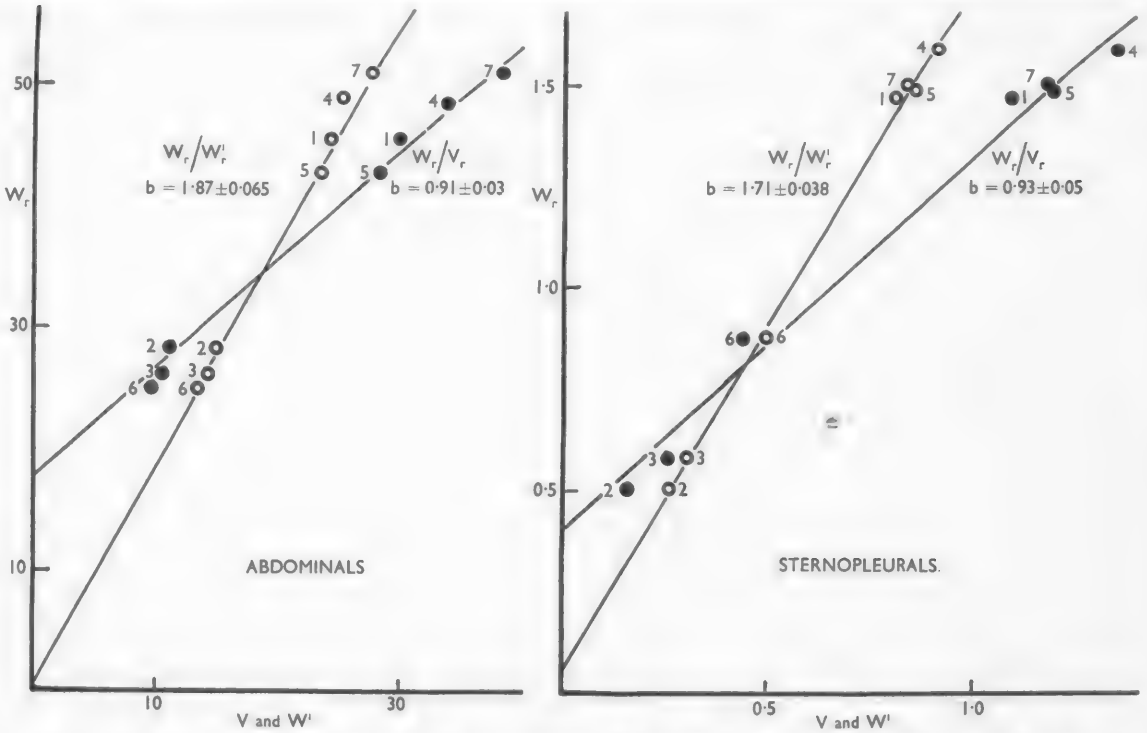

FIG. 2.—The W/V and the W/W¹ graphs from the diallel analysis of abdominal and sternopleural chaeta numbers in the first experiment. There is no evidence of marked interaction between the genes in the different segments. The numbers indicate the chromosomes to which the points relate (see table 1).

among the seven chromosomes used in the diallel. There is in any case no evidence of any marked interaction among the genes of the different segments in producing their effects, though there is clear evidence of dominance, chiefly of the segments stemming from the L line over their alternatives from H.

The main analysis, however, was carried out in a different way. Two constants were assigned to each of the four segments in respect of each chaeta character, one representing the contribution of that segment to the additive variation in respect of the chaeta character in question and the other representing its contribution to the dominance variation, in the way described by Mather (1949). These additive constants were denoted by d_a, d_b, d_c and d_d, and the dominance constants by h_a, h_b, h_c and h_d, where the subscripts a-d refer to the four segments so lettered in table 1. Since the segments are not distributed orthogonally among the chromosomes used in the experiment, the least squares technique was used to make estimates of the values of these various constants from the mean numbers of chaetæ observed for the various combinations. Given these estimates, expected values can then be calculated for the mean of each combination of chromosomes and compared with the mean hair numbers actually observed. When squared and summed the differences between the expected and observed values provide sums of squares which can be used to carry out an analysis of variance testing the significance of (i) the contribution to the additive and dominance variation, (ii) any residual effects which are to be attributed to interaction between the genes of the different segments, and (iii) the variation of the effects with sexes, backgrounds and groups of chromosomes.

In general this analysis of variance proceeds along the lines described by Mather (1949) and Mather and Vines (1952), though it should be observed that the present analysis is of first degree statistics by constants which are linear measures of variation and so can take sign, while the earlier analyses were of second degree statistics by quadratic constants which of course could not take sign. Two further points require to be made about the analyses. First, three fitting operations were carried out using different numbers of constants. In the first operation only the four d constants were fitted, the sum of squares taken out being that referable to the contribution to additive variation alone. In the second, the h_b constant was fitted in addition to the four d's, thus including an allowance for dominance in region B-b which makes the largest contribution to the additive variation and which most likely included the centromere and the regions of chromosome immediately adjacent to it on the linkage map. The extra sum of squares taken out by fitting these five constants over that taken out by the four d's measures the effect of dominance in region B-b. Finally all eight constants were fitted simultaneously. The sum of squares taken out in excess of that accounted for by the five constants of the second fitting operation measures the effect of

dominance in the remaining three segments jointly. The ascription of degrees of freedom to these sums of squares is straightforward. Pooling reciprocal pairs of heterozygous combinations leaves 28 combinations, 7 homozygous and 21 heterozygous, which will provide 27 degrees of freedom for differences amongst them. Fitting four constants uses up 4 of these degrees of freedom, five constants use up 5, and eight constants use up 8. Thus the sum of squares for the contribution to additive variation found from the first fitting corresponds to 4 degrees of freedom, the additional sum of squares for dominance in B-b to 1 d.f., and the additional sum of squares for dominance in the other three segments to 3 d.f., leaving $27-4-1-3 = 19$ for the residual sum of squares depending on genic interaction between the segments.

The second point to be made concerns the calculation of the sums of squares for change in the various components of variation (the additive, dominance and residual interaction) with sex, background and group. To take variation with sex as an example, the constants can be fitted and the sums of squares for the components of variation calculated both on data pooled over sexes and on the data for the individual sexes separately. The two sums of squares for, say, the additive component from the individual sexes, when added together give a total which will include both the sum of squares for the overall additive component and also the sum of squares for difference between the additive components of the two sexes. The former item is known from the calculation based on the data pooled over sexes and the latter item can be found by subtraction. This process is, of course, exactly analogous to the calculation of "interaction" items in the computational procedure of the standard analysis of variance and has indeed been used and described fully in its present form by Mather and Vines (1952). It can be extended to calculations of the more complex variation of the components of variation with sexes, backgrounds and groups of chromosomes in a way parallel to that used in the standard procedure of analysis of variance.

This procedure covers all items in the analysis of variance except that for reciprocals. The sum of squares for differences between reciprocal crosses can be found by the procedure described by Hayman (1954*a*) for diallel cross analysis. This can be broken down into two items by Hayman's procedure, and the variation of reciprocal differences with sex, etc. can be found; but these items have not been presented separately in the present analysis as they are homogeneous with one another. There are two less degrees of freedom for reciprocals than might be expected at first sight, since two have been lost in estimating the values for the two sexes in the missing culture, already noted, by minimisation of the reciprocal sums of squares.

The eight constants are shown averaged over sexes, backgrounds and groups in table 2, the figures given being, of course, those obtained when all eight constants were fitted simultaneously to the particular

subdivision of the data in question. The analysis of variance is given in table 3 as Mean Squares for each item. These tables include results for both abdominal and sternopleural chaetæ.

TABLE 2

The constants measuring the additive and dominance contributions of the four regions to variation in the first experiment

	d_a	d_b	d_c	d_d	h_a	h_b	h_c	h_d	s.e.
Abdominals	1·63*	7·16*	1·02*	1·55*	0·55*	−2·32*	−0·17	0·75*	0·26
Sternopleurals	0·24†	1·27*	0·15	0·32*	−0·38*	−0·54*	0·07	−0·03	0·10

* $P<0.01$. † $0.01<P<0.05$.

There is clear evidence from the analysis of variance that the additive component of variation, the dominance component for region B-b and the dominance component for the other regions jointly are significant in both abdominals and sternopleurals. In the abdominals

TABLE 3

Analysis of variance of results from the first experiment

Item	N	Abdominals	Sternopleurals
Components (C)			
Additive effects	4	3434·72*	107·329*
Dominance—region B	1	466·63*	33·153*
Dominance—other regions	3	41·04*	4·959*
Residual interaction (I)	19	15·22*	0·544†
Sexes (S)	1	8085·38*	49·786*
Backgrounds (B)	1	681·07*	703·929*
Groups (G)	1	21·17†	1·157
S×B	1	76·61*	15·881*
S×C	8	16·84*	0·802*
S×I	19	4·24*	0·315
B×C	8	13·58*	3·934*
B×I	19	7·99†	0·656
G×C	8	4·80	2·911*
G×I	19	9·50†	0·884
Reciprocals	84	3·51	0·414
Error 1	146	1·99	0·290
Error 2	49	4·16	0·903

For the applicability of the two error variances, see text.

* $P<0.01$. † $0.01<P<0.05$.

the item for residual interaction is also significant, so that in respect of this character the genes in the different segments clearly show some interaction in producing their effects. This item for interaction is less clearly significant in the sternopleurals but even here it is at least suggestively large.

Turning next to sexes, backgrounds and groups, a caution must be introduced immediately. The sex difference and its relation to

the components of variation is measured by comparisons within each of the four parts of the experiment distinguished by background and group. The effects of these latter were, however, found by comparison between these four parts. As already noted these four parts were carried out on different occasions and so may show differences also as a result of any changes which may have occurred in the environment. Thus different estimates of error variation must be used in testing the relations of components to sex and of components to background and group. The error variance for testing the sex difference and the items showing its relation to components, background groups and so on may thus be found from the triple interaction (in the statistical sense) between components, sex and background and components, sex and groups, and from the quadruple interaction. These are similar in value to one another and are pooled as error (1) in the table. The error variance for the items relating backgrounds and groups to components is to be found from the triple interaction of components, backgrounds and groups shown as error (2) in the table. The main items for background and groups should strictly be tested against the sum of squares for their statistical interaction, but this has only 1 degree of freedom and so is of little use as an error variance. Its mean square of 6·5 for abdominals and 0·990 for sternopleurals is close to that of error (2) which has therefore been used in its place and in which it is indeed included.

The sex difference in abdominals requires no testing: it is a striking feature of the results and is already well known. When tested against the appropriate error variances it is clear that the genetic background has a marked effect on abdominal chaeta number. There is a good indication, too, that the mean square from groups, with a probability of about 0·02, is significantly large. Evidently similar chromosomes constructed on different occasions are not exactly alike in their genic contents, doubtless because of differences in the position of crossing-over within the marked segments. Continuing down the analysis of variance, the sex difference clearly varies with the background (item S $\times$B), and the additive and dominance components vary significantly in value with sex (S $\times$C). There is also evidence that the residual genic interactions vary with sex (S $\times$I), though perhaps to a lesser extent. The additive and dominance components vary also with background (B $\times$C) but there is no evidence of their variation with group (G $\times$C). The residual genic interaction appears to vary with both background (B $\times$I) and group (G $\times$I). This last result suggests that components, too, may well vary with group even though this present analysis has failed to reveal it. It is noteworthy that the mean squares for residual interaction and for the variation of components with sex and background are of closely similar magnitude. Since S $\times$C and B $\times$C test the relation of the components springing from genes in chromosome III with those of other chromosomes, they too depend on genic interaction. The indications are

thus that the interaction of genes within chromosome III are of the same order of effect on abdominal chaeta number as the interaction of genes in different chromosomes. There is no good evidence of differences between reciprocal heterozygotes.

The additive and dominance components can be investigated further by considering the values of the constants which were fitted. Table 2 sets out the estimated values of the four d and the four h items for both abdominals and sternopleurals. The eight parts of the experiment distinguished by sex, background and group each yielded a value for d and h. The values shown are means taken over the eight parts. These are tested for departure from zero, the value expected in the absence of genetic effect, by comparison with a common standard error based on the differences among the eight estimates of each constant. Each constant provides seven such differences so that the overall standard error appears to be based on $7 \times 8 = 56$ degrees of freedom. This is, however, an overestimate of the number of degrees of freedom, since not all of the 56 comparisons are fully independent of each other. It is, however, hardly worth the attempt to allow for this dependence, as even only half this number of error degrees of freedom would be sufficient to give a good test of the constants.

For the abdominal chaetæ each of the four d's is significantly larger than zero, and each is positive showing that, as might be expected, the segment derived originally from the H line has a higher chaeta-producing effect than its alternative from L. Thus all segments are genically active but B-b contributes more to the additive variation than do all the other three segments put together. The dominance effects are smaller, but again all but h_c are at least suggestively large, that from B-b being once more especially striking. Not all the dominance is in the same direction.

The segment B-b also has the greatest effect on sternopleural number in both d and h. D-d makes a clear contribution to the additive variance and A-a a very suggestively large one, but there is no good evidence of an effect in C-c. A-a makes a significant contribution to the dominance component of variation in sternopleurals but C-c and D-d show no clear effect on dominance. Again the h items vary in direction of the dominance they reveal.

3. THE SECOND EXPERIMENT

This was designed as a set of diallel crosses involving eight chromosomes, *viz.* (3), (5), (6), (8), (9), (10), (11) and (12) of table 1. The diallel was set up twice, the background being M in both cases. Each diallel, however, had its own group of chromosomes, the two groups being as before similar but non-identical in that they were constructed on different occasions. Only abdominal chaetæ were counted.

Both diallels ran into difficulties. In the first diallel, infertility of females heterozygous for chromosome (12) resulted in five combinations receiving this chromosome from the mother failing to appear. In the second diallel the combination homozygous for chromosome (9) failed because of a lethal mutation, the culture which should have given combination (6) (9) failed completely and all flies carrying combinations which included chromosome (11) had to be rejected because of a major mutation which this chromosome (but not its counterpart in the first diallel) proved to carry. When homozygous this mutation reduced the number of chaetæ by an average of 19·1 as compared with its counterpart in the first diallel of this experiment, and when heterozygous by an average of 9·7. It and its normal allele appear therefore to show no dominance in respect of one another. It will be noted that the mutation appeared in a chromosome with recombination between regions P and Q, *i.e.* very close to the centromere and possibly in the heterochromatin. One wonders, therefore, whether this change could have been the direct result of crossing-over in a place where it very rarely occurs. Crossing-over can bring about lethal mutations in the second chromosome (Wigan, 1949*b*) and has been suspected as a cause of visible mutations, including an incompletely dominant one (Mather and Harrison, 1949). However this may be, the combination containing this chromosome clearly had to be omitted from the analysis of the results.

Both diallels were thus incomplete to an extent which precluded useful reconstruction by the missing-plot technique. No attempt has been made therefore to carry out any of the normal kinds of diallel analyses. Instead eight constants, four d's and four h's, have been fitted to the results separately for males and females in the two parts of the experiment. It will be seen that although five regions, A-a, P-p, Q-q, R-r and D-d are varied among the chromosomes of this experiment, A is always accompanied by d and a by D. The constants we can find are thus d_{a-d}, d_p, d_q, d_r, h_{a+d}, h_p, h_q, and h_r, where d_{a-d} and h_{a+d} measure the joint effects of substituting A+d for a+D. The values found from the results are shown in table 4.

The values of the constants found from the two sexes in the same diallel agree quite well, but the two diallels show strikingly different values for several of the constants. Evidently here, to an even greater extent than in the first experiment, the genetical activity we can attribute to the chromosome regions we recognise is being affected by either the precise circumstances under which the diallel was carried out, or more likely by the precise positions of the points of recombination. The relations that the changes in the regions P, Q, R bear to each other support the attribution of the differences to variation in the points of recombination. The genetical activity of each region changes materially between the diallels, yet their total activity, $d_p+d_q+d_r$, is virtually constant, being 8·12 and 8·06 in the two diallels respectively. The sum of the three h's is also virtually the same in the two diallels

(table 4), though here the three individual items change but little. Too much weight must not be placed on this evidence, as in the present experiment there is a strong negative correlation between the estimates of the constants attaching to the different regions, and especially between d_p and d_q. For what it is worth, however, the evidence

TABLE 4

A. *Additive and dominance components of variation in the second experiment. Abdominals only*

	d_{a-d}	d_p	d_q	d_r	h_{a+d}	h_p	h_q	h_r	s.e.
1st Assay									
Males	1·69	−0·75	6·26	1·86	0·57	0·27	−1·79	0·05	...
Females	0·53	−0·42	6·42	2·87	1·56	−0·22	−1·30	−0·44	...
Mean	1·11*	−0·58	6·34*	2·36*	1·07*	0·02	−1·55*	−0·19	0·36
2nd Assay									
Males	−0·18	1·24	4·88	1·40	0·61	−0·25	−1·35	−0·24	...
Females	−1·65	2·46	4·49	1·65	1·01	−0·34	−1·31	−0·44	...
Mean	−0·91†	1·85*	4·69*	1·52*	0·81*	−0·29	−1·33*	−0·34	0·36
Grand Mean	0·10	0·63†	5·51*	1·94*	0·94*	−0·13	−1·44*	−0·27	0·25

* $P<0·01$. † $0·01<P<0·05$.

B. *Comparison of constants from 1st and 2nd experiments. Abdominals only*

	d_{a-d}	h_{a+d}	d_b+d_c*	h_b+h_c*
1st experiment				
1st group	−0·01	0·96	7·92	−2·78
2nd group	0·16	1·66	8·45	−2·19
Mean	0·08	1·31	8·18	−2·49
2nd experiment				
1st assay	1·11	1·07	8·12	−1·72
2nd assay	−0·91	0·81	8·06	−1·96
Mean	0·10	0·94	8·09	−1·84

* Regions B+C = P+Q+R.

suggests a virtually constant amount of genetic activity being subdivided in different ways in the two diallels, as would be expected from variation in the points of recombination.

A different subdivision of this portion of the chromosome can be seen in the first experiment, for it will be recalled that regions P, Q and R jointly cover the same length of chromosome as do regions B and C. None of the individual regions match in respect of genetic activity in the two experiments and there is no reason why they should, but the total again agrees well in respect of both d and h

activity (table 4 B). The fact that d_c is less than d_r, although region R presumably forms but part of C (fig. 1) suggests that despite the selection practised on the parental H and L lines, there is still a modicum of balancing in this part of the chromosome, C carrying some genes tending to reduce chaeta number and c some tending to increase it. The same is suggested by the negative value for d_p in the first diallel, though admittedly this is not a fully significant departure from zero. The values for d_{a-d} and h_{a+d} when compared over the two experiments are more variable than the sum of activities of the middle portion of the chromosome. This is perhaps understandable of a difference depending on two regions both long on the genetical map and delimited by points of recombination which vary simultaneously and independently of one another.

One further point remains to be made about the results of the second experiment. With the exception of d_p in the first diallel all the d values depart significantly from zero, so that there is evidence of every region being active. In neither diallel do h_p and h_r depart significantly, but h_{a+d} and h_q are significant in both.

4. THE THIRD EXPERIMENT

This, too, consisted of two diallels, the first including the six chromosomes (1) and (13)-(17), and the second the six chromosomes (2) and (18)-(22) as listed in table 1. Thus both can provide information on the activities of regions X-x, Y-y and Z-z, but the effects of regions A-a, D-d and R-r contribute to the overall difference between the diallels and so are confounded with one another, with any effect of difference in the precise points of recombination delimiting regions X, Y and Z and with any difference of environmental effects between the two diallels. It is thus profitless to discuss anything but the overall effects of X, Y and Z in this experiment.

In the first diallel five cultures failed. The mean number of abdominal chaetæ were taken as equal to those from the reciprocal crosses in four cases which would have yielded heterozygous combinations. For the abdominals in the fifth case (which would have given a homozygous combination), and for the sternopleurals in all five cases mean counts for flies raised specially on a different occasion were used to complete the diallel table. No culture failed entirely in the second diallel though in a few cases here, as in the first one, less than the usual 10 flies appeared. The mean count had then to be based on fewer than the customary number.

An analysis of variance of the results was carried out similar in principle to that in the first experiment. No attempt was made, however, to separate the sum of squares due to dominance from that due to the additive variation, the six components (d_x, d_y, d_z and h_x, h_y, h_z) being fitted simultaneously. Furthermore, as has been seen, the difference between the diallels could not be ascribed simply

to difference in background, or group of chromosomes. The results of the analysis of variance are shown as mean squares in table 5, their most striking feature being the close similarity they bear to those of the first experiment. Taking the abdominals first, the mean square reciprocal difference is 3·22 as compared with the earlier figure of 3·51. Not being significant, this item was pooled with the high order interaction to give an error variance of 2·96, which is again closely similar to that of the earlier experiment.

TABLE 5

Analysis of variance of results from the third experiment

Item	N	Abdominals	Sternopleurals
Components (d and h) (C)	6	430·25*	14·84*
Interaction (I)	14	10·93*	0·55
Sexes (S)	1	4305·55*	34·42*
Assays (A)	1	464·40*	46·69*
S×A	1	21·31*	0·75
A×C	6	19·78*	0·90*
A×I	14	6·19†	1·59*
S×C	6	5·94	0·33
S×I	14	3·70	0·07
S×A×C	6	3·23	0·18
S×A×I	14	1·89	0·10
Reciprocals	52‡	3·22	0·28
Pooled Error	72‡	2·96	0·24

* $P<0.01$.

† $0.01<P<0.05$.

‡ For sternopleurals N = 58 and 78 respectively—see text.

Once again, not only the item for components of variation, but also that for residual interaction is significant, being in fact of the same order of size as in the first experiment. Furthermore the interaction of components with sex and diallels at an average of 12·85 is much the same as the corresponding average (of B×C, G×C, and S×C) which was 11·74 in the first experiment. Thus once again the genic interaction within chromosome III is of the same size as the interaction between this chromosome on the one hand and its fellows and environmental differences on the other. There is some hint again of the interaction within the chromosome varying with the background.

Much the same can be said of the genic activity as expressed in the number of sternopleural chaetæ, the chief difference from the first experiment being that the interaction of components and other effects is now more nearly like the residual interaction itself in size.

The estimated values of the actual components of genic variation are set out in table 6. The total effects on the abdominals (d_{x+y+z}

and h_{x+y+z}) vary somewhat between the two diallels, but the average additive effect of the two diallels at 6·53 agrees well with the sum (6·15) of the effects of regions p and q, which cover the same length of chromosome in the second experiment. No similar comparison is possible for the sternopleurals which were not followed in the second experiment. The individual components significantly demonstrate action only in region Y-y, but the significant item for interaction remaining

TABLE 6

A. *Additive and dominance components of variation in the third experiment*

Averaged over sexes	d_x	d_y	d_z	h_x	h_y	h_z	s.e.
Abdominals							
1st Assay .	0·82	6·18*	1·13	−1·06	−2·34*	0·45	0·53
2nd Assay .	0·49	4·65*	−0·20	−0·21	−1·42†	−0·22	0·53
Mean . .	0·66	5·41*	0·46	−0·64	−1·88*	0·12	0·37
Sternopleurals							
1st Assay .	−0·19†	1·39*	0·01	−0·15	−0·13	0·08	0·08
2nd Assay .	−0·15	0·84*	0·09	−0·15	−0·40*	0·05	0·08
Mean . .	−0·17†	1·11*	0·05	−0·15†	−0·27*	0·06	0·06

* $P<0·01$. † $0·01<P<0·05$.

B. *Comparison of constants from second and third experiments*

	2nd experiment		3rd experiment	
	d_{p+q}	h_{p+q}	d_{x+y+z}	h_{x+y+z}
1st Assay . .	5·76	−1·53	8·12	−2·96
2nd Assay . .	6·54	−1·62	4·94	−1·85
Mean . .	6·15	−1·58	6·53	−2·40

in the analysis of variance after fitting the individual components must be taken as evidence that even so regions X-x and Z-z nevertheless share some activity since they (or at least one of them) interact in effect with region Y-y.

5. THE FOURTH EXPERIMENT

The design of the earlier experiments was such that the effects of the different regions of the chromosome were not orthogonal to one another with the consequence that they could be assessed only by the successive fitting of constants. This last experiment utilised eight chromosomes representing every combination of the three compound regions A-a, PQ-pq and RD-rd. The effects of these compound regions and also their interactions can thus be assessed individually

by an analysis of variance using the customary orthogonal functions. Only homozygous combinations were used and only d components of variation can therefore be estimated. Two groups of such chromosomes, constructed separately, were used and the whole experiment duplicated, so that there were in effect four observations of each genotype. No combination failed in all four replicates, but a few

TABLE 7

A. *Analysis of variance and covariance—fourth experiment*

Item	N	Abdominals	Sternopleurals	Stps. corrected for regression on abds.
Additive effects (C)	3	1028·78*	41·83*	21·91*
Interaction (I)	4	2·80	0·30	0·27
Sex (S)	1	1718·10*	24·63*	0·16
S×C	3	10·17*	0·48	0·32
S×I	4	4·12†	0·89†	0·85†
Error (between cultures)	20	1·40	0·56	0·59
Error (within cultures)	20	1·45	0·21	0·22

B. *Additive components of variation—fourth experiment*

	d_a	d_{p+q}	d_{r+d}	s.e.$_{[40]}$
Abdominals				
Males	0·45	6·14	1·28	...
Females	0·25	7·39	1·83	...
Average	0·35†	6·76*	1·55*	0·15
Sternopleurals				
Males	1·02	0·74	0·33	...
Females	1·09	1·02	0·22	
Average	1·06*	0·88*	0·27*	0·09

s.e. derived from the pooled error items.
* $P<0·01$. † $0·01<P<0·05$.

failed in some of them. Thus there are fewer degrees of freedom for error than would otherwise have been the case.

The analysis of variance (table 7) shows the error variation to be lower for abdominals in this than in the earlier experiments, that for sternopleurals being much as before. The main d components are significant for both characters, as is the sex difference. Interactions of activity of the three compound regions are significant for neither character, though the sex-component interaction is significant in the case of the abdominals. While this experiment therefore fails to repeat the earlier ones in showing clear interactions within the chromosomes, it fully supports them in showing that whatever interactions of

this kind there may be they are certainly no larger than those between chromosomes. There is again no evidence of any special interaction within the chromosome.

The value of d_a is somewhat smaller for abdominals and larger for sternopleurals than had been found earlier, but the other d values were of about the size expected from earlier experience.

An analysis of covariance was carried out to test the degree of dependence of variation in sternopleurals on that in abdominals. The whole of the sex difference in sternopleurals can be accounted for by relation to the sex difference in abdominals, but only half the effect of the three d components can be taken up in this way. The genic activity of the third chromosome is thus not equivalent for these characters. Some genes may affect both characters similarly, but the gene systems controlling them are certainly not identical : the characters are capable of independent genetic adjustment at least in part.

6. DISCUSSION

In the first experiment, chromosome III was subdivided into four regions all of which proved to be genetically active in their effects on the number of abdominal chaetæ, so requiring the postulate of at least four genes. In the second experiment the minimum of genes was increased, since two of these three regions were jointly subdivided into three, all of which again proved to be active. Furthermore, although the total activity of regions P, Q and R appeared constant over the two parts of this experiment, its distribution between the three regions differed in the two parts of the test. The precise point of recombination is thus important in determining the distribution of activity between the regions, so that a minimum of three genes between these three regions would appear insufficient to account for the observation. The second experiment therefore raises the minimum necessary in the whole chromosome to more than five, though whether to six or seven depends on a more detailed and therefore more debatable interpretation of the second experiment. In the third experiment regions P and Q were again jointly subdivided into three, but this adds little to our information as only one of the new regions, Y, shows marked activity, the evidence of activity in X and Z being inconclusive.

It is important, too, that apart from the last subdivision not merely did each fractionation of the chromosome reveal more genes, but every region proved to be active. It is unlikely therefore that the minimum number of six or seven would not be raised still further by further analysis of the chromosome. We are in fact led to picture the chromosome as being genetically active in respect of abdominal chaeta number along the whole or at least great parts of its length ; to picture it as being in other words truly polygenic in its activity. The activity of any region of the chromosome is then not an attribute of a single gene but of that constellation of genes which the region

happens to contain. The region is to be regarded as an effective factor for the purposes of the experiment, and a factor which could be further broken down when the region is fractionated by further recombination. Regions B, Q and Y admittedly stand out from the rest by reason of the great activity which they display relative to their lengths on the genetical map, so suggesting a major concentration or even a single locus of major effect. Reference to fig. 1 shows, however, that B and Y certainly include the region of chromosome near the centromere, while either P or Q must also do so. Now recombination is rare near the centromere so that the genetical map greatly underestimates the physical length of chromosome regions in this neighbourhood. Indeed if we seek to relate activity to length in the mitotic chromosome, the high activity of these centromere regions ceases to be noteworthy. We can thus understand the distribution of activity between the various regions if we regard the polygenic system from which the activity springs as distributed roughly evenly along the physical length of the chromosome. Such an approximately even distribution would not be surprising, and indeed might even be anticipated given that any sizeable block of heterochromatin near the centromere is, mitotic length for length, about as polygenically active as the euchromatin of more distal regions.

It has sometimes been proposed (*e.g.* by Reeve and Robertson, 1953) that far from such continuous variation being mediated by polygenic systems, it is governed by a few loci of major effect, the continuity of variation springing from the existence of a range of so-called "iso-alleles" at each locus. This postulate meets with considerable difficulty in explaining even the results of selection experiments for which it was proposed (Mather, 1954): it is impossible to reconcile with the results of the present survey.

There might still be some justification for treating the chromosome, or some large section of it, as a unit (though it could not be called a gene without such a gross straining of the term as to render it meaningless) if the genes within it displayed some unique form of interaction peculiar to them because of their physical association. Such interaction, depending on special physical relations of the interacting entities, must be expected within very short regions of chromosomes and serve to define genes (Mather, 1946, 1954; Goldschmidt, 1951); and they are indeed known in the phenomenon known as pseudo-allelism. There is, however, no trace of them over the longer lengths of chromosome with which we are dealing. The genic interactions detectable within chromosome III seems to be no greater than, and no different from, those between the genes of this and other chromosomes.

There is good evidence that the genes in several of the segments show dominance. In the regions B, Q and Y the heterozygous combination is nearer to the combination homozygous for the group of genes from the L parent. The h component of activity is nevertheless

lower in value than the d, so that either the genes in question are only partly dominant or there exist within the group some genes with dominance in the opposite direction despite the overall preponderance of dominance for the lower chaeta number. There is also a suggestion of dominance in the low direction of genes within the X segment and of dominance in the high direction of those in A and D. Thus, as would be expected in a polygenic system (Mather, 1943), dominance is not completely unidirectional though it is of interest that again, as Fisher (1930) has suggested would be the case, it is preponderantly in the direction away from the heavier and more effective selection, chaeta number of the H line having been raised more by the selection practised in its production than L was lowered.

Turning to the sternopleural chaetæ we note that a number of the segments show genic activity in respect of this character, too, and indeed the situation is very much the same as for the abdominals. That the differences in activity between the pieces of chromosome derived from H and L are relatively not so great as those in respect of the abdominals is suggested by two comparisons. In the first place, the ratio borne by the components' mean square to the error mean square is higher for abdominals than sternopleurals in every analysis of variance and markedly higher in some. And secondly, the d components for the sternopleurals are lower relative to the overall mean number of these chaetæ than for the abdominals. Segments B, Q and Y show the greatest activity in respect of sternopleurals as in respect of abdominals, but this is to be expected if the large effect is due to the great physical length of these regions. Otherwise there is little evidence of a correlation between sternopleural and abdominal activity. In other words, we have no grounds for believing that the sternopleural differences reflect merely a pleiotropic activity of the gene system governing the number of abdominal chaetæ, and the analysis of variance in the fourth experiment bears this out. The occurrence of some pleiotropic activity cannot be ruled out, and Cocks (1954) has indeed obtained some evidence of it ; but the characters are evidently controlled by at least partly different genic systems so that they can be adjusted separately by selection. The correlated responses of sternopleural chaeta number to selection for abdominals would then be in the main due to the linkage relations of the polygenic systems controlling them, as has been amply demonstrated (Mather and Harrison, 1949) for this and other characters in earlier selection experiments.

7. SUMMARY

The distribution of genetic differences between the third chromosomes of two selected lines of *Drosophila melanogaster* was investigated in respect of number of abdominal chaetæ (for which the selection has been practised) and number of sternopleural chaetæ. By means of a marker stock, recombinant chromosomes were constructed

which consisted of known portions of the two selected third chromosomes but which themselves carried no marker genes. The six regions into which the chromosome was thus divided were tested for contributions to additive variation, for dominance and for genic interaction, and the variation of these effects with sexes and with genetic background was observed. All regions showed genetic activity and most showed dominance effects. Genic interaction was detected within the chromosome but it appeared to be of no greater effect than the interaction of genes in the third chromosome with genes in other chromosomes. The activity was greatest in the region of the centromere when related to the genetical map, but appeared to be more or less evenly distributed along the chromosome when related to the physical lengths of the regions.

At least six genes must be involved in the third chromosome's contribution to the differences between the two selected lines, and the data are most readily interpreted in terms of a polygenic system spread along the whole chromosome. They cannot reasonably be reconciled with the postulate of a very few loci of major and complex effect each varying to give a range of iso-alleles. Though some genes may affect abdominal and sternopleural chaetæ simultaneously, the genetic systems governing the two are at least in part distinct from one another. The characters can thus be adjusted separately by selection. The correlated responses in the one to selection for the other must depend in the main on the linkage relations of the systems rather than pleiotropy of gene action.

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DETERMINATION OF SEX THROUGH GENES IN A MAJOR SEX LOCUS IN *DROSOPHILA MELANOGASTER* *

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HEREDITARY control of sex had first support when unequal chromosomes were shown to be directly correlated with the sex of an individual or its progeny. Over the years no specific sex loci could be located within these chromosomes, however. Polyploid types within *Drosophila* showed that the sex chromosomes were not the only factors. Sex determination appeared to rest rather on a balance between the sex chromosomes and the autosomes. In *Drosophila* the X chromosomes operated by pushing the organism toward the female type, the autosomes pressed toward the male type. The Y chromosome had little effect. Loci for major sex genes utilising the intersex types as test agents were sought but again in no clear-cut instance were they found. In the interim major sex genes appeared through observed mutational types.

Sturtevant (1920, 1921) in *D. simulans* isolated a gene in the second chromosome which could convert diploid females to intersexuals, and rendered males sterile. Phenotypically these intersexuals were female-like in that they lacked sex combs, had 7 dorsal abdominal tergites, ovipositor of abnormal form, 2 spermathecæ and lacked the penis. They were male-like in having first genital tergite although abnormal in form, lateral anal plates, claspers, black pigmented tip to the abdomen. The gonads were rudimentary. The gene, as was expected since it was recessive, had no effect on *D. simulans* × *melanogaster* hybrids.

Lebedeff (1934) in *Drosophila virilis*, studied a recessive gene in the third chromosome which converted diploid females into intersexuals. These intersexuals were arranged on the basis of their morphology from extreme female type with nearly normal female appearance and reproductive organs (ovary undifferentiated) to male type (undeveloped testis and sperm). Between these types numerous hermaphroditic progeny occurred having imperfect but corresponding male and female organs jointly present.

Dobzhansky and Spassky (1941) in *Drosophila pseudoobscura* observed 2 progenies containing intersexual individuals. These intersexuals were thought to be due to a single dominant gene transforming diploid females. They were characterised by two sets of more or less complete genital ducts and external genitalia but only one pair of gonads. One set of ducts and genitalia was almost always more female-like

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and the other more male-like. Sex combs were present. The distal comb had 2 to 4 and the proximal 4 to 6 teeth, as compared with the normal male number of 4 to 7 on the distal and 6 to 9 on the proximal combs. Body development was more like the female.

Briles established a dominant gene which caused intersexuality in diploid females. Stone located this gene in the second chromosome of *Drosophila virilis*. Newby (1942) presented an extended study of the embryological sequence in development of the organs of these intersexual types. Externally, these intersexuals have a total of 9 tergites. The first 6 of these tergites were like those in the normal female, and the last 3 were small and irregularly formed. Six sternites were present; the first 5 were normal but the 6th was malformed. The anal valves were lateral as in the male, but a 3rd small valve was also present at the ventral side of the anus. Clasper plates of irregular pattern were found ventral to the anus. Below the clasper plates, the vaginal plates were often extruded as a genital knob. This knob occasionally became heavily pigmented. Internally, development ranged from nearly female individuals through the hermaphrodite type containing representative organs of both sexes to individuals almost wholly male. In brief, from these intersexuals, Newby concluded that "intersexuality expresses itself as a response to the developmental pressure of both sexes, not as development in one direction followed by a change" (page 139).

These cases were for single mutant genes in which there was no sure method of showing that the wild-type gene truly operated on the sex embryology and phenotype.

Gowen in 1940 established a stock carrying the dominant gene in *Drosophila melanogaster*, *Hr*, which affects diploid females; producing sterile individuals with complete male secondary reproductive systems associated with their female counterparts. The primary gonads were certainly often ovarian but in rare instances had a crude resemblance to testicular tissue. This gene showed a dosage effect in triploids but no effect on males. Dosage effect in triploids in conjunction with the types found in the diploids showed that both the mutant, dominant gene *Hr*, and its wild-type counterpart influenced both the embryological and phenotypical sex. In consequence both the *Hr* and *hr*$^+$ may be regarded as true major sex genes (*hr*$^+$ is hereafter written as simply +). Sturtevant (1945) established a recessive gene "*tra*" in *D. melanogaster* which was also sex limited in effect. When homozygous, the gene transformed diploid females into sterile males. The primary and secondary sex organs of these "males" were so phenotypically like normal males as to be nearly indistinguishable from them. This gene also changed sex in certain of the polyploid types. In recent crosses we have shown that these three genes +, *Hr* and *tra* behaved as an allelic series in the sense that crosses between them showed distinguishable phenotypes when such differences would be unlikely if the genes were not allelic or pseudo-allelic. Possible

combinations of these genes in XY+2A, XX+2A and XXX+3A flies resulted in some 10 distinguishable sex types. The descriptions and implications of these sex types are considered in the rest of this paper. The problem of what happens in intersexes XX+3A, super females XXX+2A and super males XY+3A will be discussed at a later date.

1. MATERIALS AND METHODS

The gene series is located in the third chromosome of *D. melanogaster*. Crosses to form diploid and triploid combinations of the + and *Hr* genes were made several years ago (1942) and were again represented with like results. Sturtevant kindly supplied the *tra* gene. The 3 genes have been crossed to form all +, *Hr* and *tra* combinations possible in diploids and triploids. Where comparable these crosses gave phenotypes which confirmed those described by Sturtevant or ourselves.

2. EXPERIMENTAL RESULTS

The descriptions of the different recognisable sex phenotypes are summarised in table 1 for the XY+2A and XX+2A, diploid and XXX+3A triploid types.

The reproductive systems differentiating males and females in *Drosophila* are as complex as those in any animal. It is evident that the three genes under study can convert *Drosophila* from a bisexual species into multisexual phenotypes. The new forms of especial interest may be described and illustrated as follows.

Hr/+ (diploid hermaphrodite). Externally the body size and form of the hermaphrodite flies resembled diploid females. They had sex combs on the basal tarsal segment of each front leg. The comb consisted of 6-8 slender teeth instead of the usual 10 or more found in normal males. The anal valves were of male type but were often displaced and asymmetrical in arrangement. Between the anal valves was a pair of abnormal claspers thickly sclerotised and supported by irregular plates. Within the clasper, orange penis parts appeared. Beneath these plates, a heavily-pigmented and sclerotised protuberance was ordinarily found. This knob generally contained the spermatheca and represented the posterior part of the female genitalia. These flies were sterile.

The internal genitalia of the hermaphrodites exhibited great variability. In the array of individuals studied, there was not a single case which showed complete sex reversion to male type. Each specimen consisted of a mixture of either well-developed or rudimentary sexual organs. Some had well-developed ovaries, in others these organs were rudimentary but all flies of this type contained distinct sexual organs of either sex. In most individuals, tiny yellow masses were found to be associated with the gonads or the genital systems, particularly the paragonia. The pigmentation of these tissues resembled the yellow pigment present in the testicular epithelial sheath cells. The male accessory glands, the paragonia, were most commonly to be observed. They were irregular in form, number,

TABLE 1

Graphical description of sex phenotypes found in 16 different sex genotypes in Drosophila

IIIrd chromosome genotype	XY+2A	XX+2A				XXX+3A				
	+/+, +/*Hr*, +/*tra*, *Hr*/*tra*, *tra*/*tra*	+/+ +/*tra*	+/*Hr*	*Hr*/*tra*	*tra*/*tra*	+/+/+ +/+/*tra*	+/+/*Hr*	+/*Hr*/*tra*	*Hr*/*tra*/*tra*	*tra*/*tra*/*tra*
General size	♂	♀	♀	♀	♀	♀	♀	♀	♀	♀
Phenotypic sex	♂	♀	♂ and ♀	♂-	♂	♀	♀-	♂ and ♀	♂	♂
Sex combs	10-12	...	6-8	8-9	9-12	...	4-6	5-7	8-9	9-11
Anal plates	♂, vert.	♀, trans.	♂, ♀	♂	♂	♀	♀	♂	♂	♂
Claspers	+	−	±	−	+	−	−	±	+	+
Penis	+	−	±	−	+	−	−	±	+	+
Ovipositor	−	+	+	−	−	+	+	±	−	−
Ejaculatory duct	+	−	+	+	+	−	−	±	±	+
Oviduct	−	+	+	−	−	+	+	−	−	−
Sperm pump	+	−	±	+	+	−	−	±	+	+
Ventral receptacle	−	+	+	−	−	+	+	−	−	−
Spermatheca	−	2	+	−	−	2	2±	−	−	−
Parovaria	−	2	+	−	−	2	2	−	−	−
Paragonia	2	−	+	+	+	−	−	±	+	+
Vasa deferentia	2	−	±	+	+	−	−	±	+	+
Testis	2	−	−?	±	±	−	−	−	±	±
Ovary	−	2	±	±	−	2	2	+	−	−
Fecundity	+	+	−	−	−	+	−	−	−	−

and attachment. The sperm pump was likewise abnormal in shape. The rod-shaped sclerite at the centre of the pump was generally missing or, if present, poorly developed. Of the female accessory organs, the spermathecæ and ventral receptacle were generally present. Dual duct systems which contained both ejaculatory ducts and oviducts were found in most individuals. Structural differences in these types and the behaviour of the imaginal discs for these organs on transplantation have been extensively studied (Fung and Gowen, 1957*a*, 1957*b*).

Hr/tra. The body size of these flies resembled that of females. Morphologically, this sex type was clearly distinguishable from the *Hr*/+ hermaphrodites. Their external genitalia were male-like but appeared retracted, with no visible claspers. On the first tarsal segment of the forelegs, the sex combs consisted of a row of 8-9 teeth.

The internal genitalia were generally composed of male sexual organs. Vasa deferentia and paragonia as well as the ejaculatory ducts and sperm pump were well developed. Gonads generally consisted of rudimentary or under-developed testes. The development of the female genital system seemed to be almost replaced by that of the male.

Hr/+/+. Externally these flies resembled +/+/+ triploid females. They normally had larger bodies, coarser bristles, and larger ommatidia and wing cells than diploid females of XX+2A, +/+ genotype. They were distinguished from +/+/+ by having sex combs of 4-6 long and slender teeth. The tips of their abdomens were slightly darkened. The internal genitalia were completely female. In spite of the great similarity between these triploids and the normal diploids, they were completely sterile.

Hr/tra/+. These flies had bodies, ommatidia, and wing cells of larger size than wild diploid females of XX+2A genotypes. Their external genitalia resembled *Hr*/+ (diploid hermaphrodites) with rudimentary claspers and sclerotised protuberances. They had sex combs on the forelegs with 5-6 teeth. The internal genitalia were predominantly male with well-developed ejaculatory ducts and paragonia. The gonads consisted of free ovaries or ovary attached to the vasa of the male genital system.

Tra/tra/tra. These individuals had large bodies, ommatidia, and wing cells. External genitalia were identical to normal diploid males. Sex combs consisted of 10-11 teeth. The internal genitalia have a well-developed ejaculatory duct, sperm pump, and accessory gland. The testes were elongated but much smaller than in normal males. These flies mated with females but were completely sterile.

3. SUMMARY

This paper presents a summary of sexual types due to the actions and interactions of three major sex genes operating on the normal

gene background of *Drosophila melanogaster*. Because of their phenotypic expression in crosses, these genes are assigned to a single major locus for sex determination in the 3rd chromosome.

Different diploid and polyploid combinations of the genes +, *Hr* and *tra* have been made for the study of their sex types. Besides the familiar normal males and females, the triploid types of Bridges, the two *Hr* types of our stock, and the two male types of Sturtevant, three new distinguishable sex phenotypes have been found. The diploid genotype *Hr/tra* had male-like but retracted genitalia and no visible claspers, sex combs of 8-9 teeth, and internal genitalia largely male. The *Hr/tra/tra* resembled the *Hr/tra*, having sex combs with 8-9 teeth, internal genitalia predominantly male, well-developed duct and accessory organs, and testes elongated slightly but much smaller than those of normal males. The *Hr/tra/+* flies had rudimentary claspers and sex combs of 5-6 teeth. The internal genitalia were developed with ejaculatory duct, paragonia and gonads with free ovaries or ovaries attached to vasa of the male system.

Besides the intersex genotype, 2X+3A, which was little affected by these genes, ten distinguishable sex types were produced by the action or interaction of these genes in the diploid and triploid flies. Dosage interactions in diploids and triploids proved that + of the wild type was a sex gene like the *Hr* and *tra*. The results show that fundamental sex characters, like other characters, sometimes may be altered by substitution of major sex genes occupying particular loci.

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THE ENUMERATION OF GENOTYPE-PHENOTYPE CORRESPONDENCES

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THE distinction between an individual's genotype, which may be determined by reference to its breeding performance and possibly that of its relatives, and its phenotype, which refers only to its observable characteristics, is fundamental in genetics. Even when only one locus is concerned, many different genotype-phenotype relationships are possible, especially if there are more than two allelomorphs. In this article, we are concerned with a problem of enumeration which arises in the classification of the genotype-phenotype relationships at a single diploid locus.

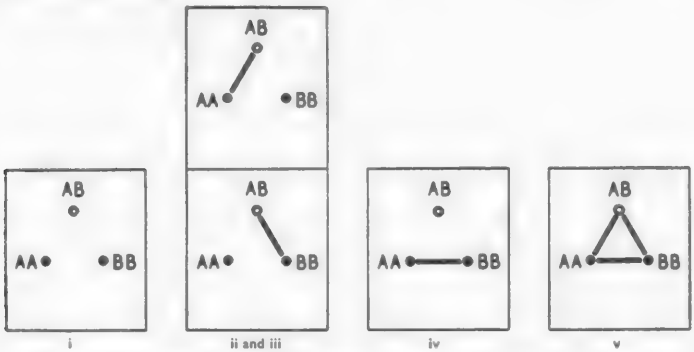

FIG. 1.—The five two-allelomorph phenotype systems classified into four phenograms (after Cotterman, 1953).

There are $\frac{1}{2}m(m+1)$ possible diploid genotypes at a single locus with m allelomorphs. We shall suppose that each of these genotypes corresponds with one and only one phenotype but more than one genotype may correspond with the same phenotype. The $\frac{1}{2}m(m+1)$ genotypes may therefore correspond with any number of phenotypes from one to $\frac{1}{2}m(m+1)$. Following Cotterman (1953), such a genotype-phenotype correspondence will be called a *phenotype system* and two phenotype systems, each involving the same number of allelomorphs and phenotypes, will be said to be permutationally equivalent or to be images of the same *phenogram* if there exist one or more permutations of the gene symbols which transform one phenotype system into the other. Thus, with two allelomorphs and three genotypes, there are five phenotype systems. Cotterman (1953) has represented these phenotype systems as shown in fig. 1, where the genotypes are represented by circles, open and closed for homozygotes and heterozygotes respectively, and any two genotypes which are phenotypically

indistinguishable are joined by a line. Fig. 1 (i) represents the case of no dominance, figs. 1 (ii) and 1 (iii) the two cases of dominance, fig. 1 (iv) the case in which the two homozygotes are phenotypically indistinguishable from each other but both differ phenotypically from the heterozygote, and fig. 1 (v) the trivial case in which all the

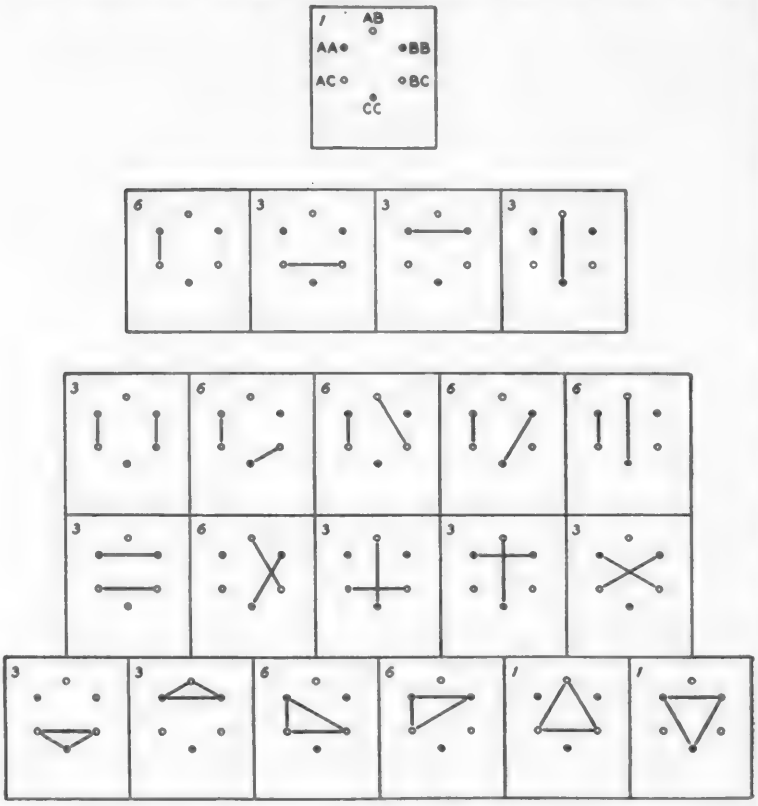

FIG. 2.—The 203 three-allelomorph phenotype systems classified into 52 phenograms (after Cotterman, 1953).

genotypes correspond with the same phenotype. Clearly, the two phenotype systems represented by figs. 1 (ii) and 1 (iii) are images of the one phenogram and there are thus four distinct phenograms in this case.

With three allelomorphs, there are six genotypes and 203 phenotype systems, *i.e.* there are 203 different ways in which these six genotypes can be grouped so as to correspond with any number of phenotypes from one to six. Cotterman (1953) has shown that in this case there are 52 phenograms (see fig. 2). With four allelomorphs

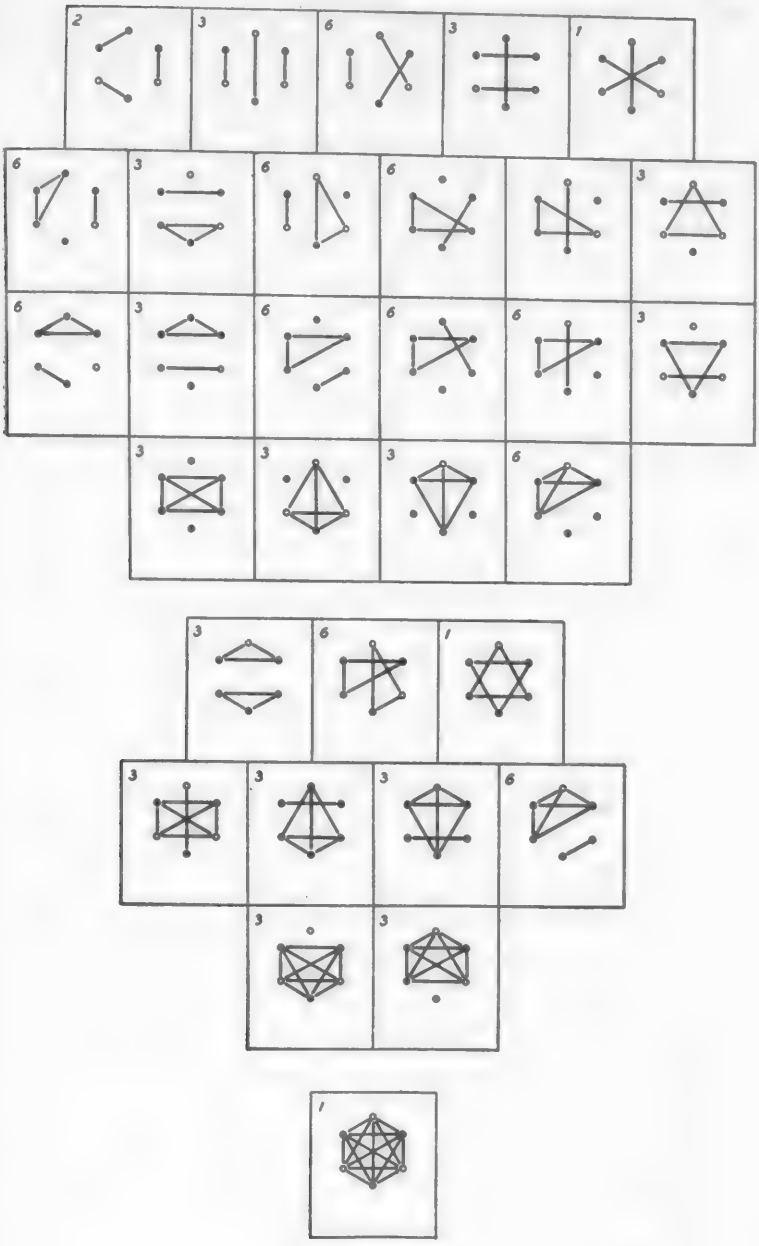

FIG. 2.—*continued*

there are ten genotypes and 115,975 phenotype systems. The phenotype systems have not been described for this case but Cotterman conjectured that the phenograms would number about 7000. With five allelomorphs, there are fifteen genotypes and 1382,958545 phenotype systems. Cotterman guessed that the phenotype systems would here belong to about 17,000000 phenograms. The exact enumeration of these phenograms presents a rather intricate combinatorial problem, and has hitherto not been described.

Each m-allelomorph phenotype system may be represented in partitional notation as a partition of the total number $G = \frac{1}{2}m(m+1)$ of genotypes. For any such partition

$$P = (p_1^{\pi_1} p_2^{\pi_2} \ldots) \text{ of } G, \text{ there are}$$

$$\beta(P) = \frac{G\,!}{(p_1\,!)^{\pi_1}\,(p_2\,!)^{\pi_2} \ldots\ \pi_1\,!\ \pi_2\,! \ldots}$$

distinct phenotype systems. This is the number of ways in which G different genotypes can be grouped into $S\pi_i = \phi$ different phenotypes according to the partition P. The total number $N_{m.\phi}$ of m-allelomorph phenotype systems in which the G genotypes correspond with exactly ϕ phenotypes is found by summing $\beta(P)$ over all partitions P with ϕ parts. The total number N_m of m-allelomorph phenotype systems is equal to $\beta(P)$ summed over all partitions P of the number G. Values of $N_{m.\phi}$ are given in table XXII of Fisher and Yates (1953), as $\Delta^{\phi}O^{G}/\phi\,!$, the leading ϕ-th difference of the G-th powers of the positive integers commencing at zero, for values of G from 2 to 25 and values of ϕ from 2 to G. Values of N_m can be obtained by summing $N_{m.\phi}$ over all values of ϕ from 1 to G.

The problem of the enumeration of the phenograms is much more difficult and belongs to a general class of enumerations which has been considered by Fisher (1950). A formula (phenotype system) is constructed by inserting appropriate " contents " into a finite number of " cells ". Any two formulæ which can be derived from one another by a permutation of the basic group of permutations among the cells are said to belong to the same set (phenogram). We wish to enumerate the number of such sets. Every permutation h of the complete permutation group of m allelomorphs produces a corresponding permutation g of the $\frac{1}{2}m(m+1)$ genotypes. If the permutation g is of partitional type Q, then of the $\beta(P)$ phenotype systems which correspond to a given partition P of $\frac{1}{2}m(m+1)$, the number which are left unaltered by the permutation g is the bipartitional function $\phi(P, Q)$ (Fisher, 1950; Bennett, 1956). Summing over all partitions P, we obtain the total number of phenotype systems which are unaltered by the permutation g as

$$\sum_{P/\frac{1}{2}m(m+1)} \phi(P, Q) = e(Q).$$

Since there are $m!$ permutations of the m allelomorphs, the total number of m-allelomorph phenograms must be

$$n_m = \frac{1}{m!} \sum_{Q} e(Q)$$

where the summation is taken over all partitions Q corresponding to the $m!$ permutations of the $\frac{1}{2}m(m+1)$ genotypes induced by all possible permutations of the m allelomorphs. Since there are

$$\alpha(R) = \frac{m!}{\rho_1!\ \rho_2!\ \ldots\ r_1^{\rho_1}\ r_2^{\rho_2}\ \ldots}$$

permutations of the m allelomorphs of partitional type

$$R = (r^{\rho}_1\ r^{\rho}_2\ \ldots),$$

it follows that

$$n_m = \frac{1}{m!} \sum_{R/m} \alpha(R)\ e(Q(R))$$

where the summation is taken over all partitions R of the number m. Values of $e(Q)$ can readily be calculated using the following operational procedure (Bennett, 1956) :—for every separation of the partition Q, construct a differential operator by forming the product of (i) $(D+f)$ for each separate with only one part and with f factors (excluding unity) in that part, and (ii) for each separate with more than one part, the sum of a^{s-1} for all factors a excluding unity which are common to the s parts in that separate. These operators are summed over all possible separations of the partition Q and are then allowed to operate on $F(x) = e^{e^x - 1}$ when they yield $e(Q)$. Since

$$D^n F(x) = e(1^n) = \sum_r \Delta^r O^n / r!,$$

this operational procedure enables us to calculate $e(Q)$ very readily if we make use of published tables of $\Delta^r O^n / r!$ (Fisher and Yates, *loc. cit.*).

The calculation of n_m for $m = 3, 4, 5$ proceeds as shown in tables 1, 2, 3 respectively. The six permutations of three allelomorphs A, B, and C, may be classified according to the partitions of the number three as shown in the first column of table 1. The partition (1^3) represents the identity which leaves the allelomorphs unchanged, (21) represents an interchange of pairs of allelomorphs of which there are three possibilities, (AB), (BC) and (CA), and (3) represents a cyclic interchange of three allelomorphs of which there are two possibilities, (ABC) and (ACB). The permutations of the six genotypes brought about by these permutations of the allelomorphs are represented in the second column of table 1. Thus if the three allelomorphs are left unchanged, so will the six genotypes be unchanged and this corresponds with the identity (1^6). An interchange of two allelomorphs corresponding to the partition (21) will lead to two possible interchanges of pairs of genotypes which may be represented by the

partition $(2^2\,1^2)$. Thus, the interchange of allelomorphs A and B will lead to the interchange of genotypes AA and BB and also of AC and BC whilst the remaining genotypes AB and CC will be unchanged.

TABLE 1

Calculation of the number of phenograms with three allelomorphs

R	Q	$e(Q)$	$\alpha(R)$	$\alpha(R)\,e(Q)$
(1^3)	(1^6)	203	1	203
(21)	(2^21^2)	31	3	93
(3)	(3^2)	8	2	16

$$n_3 = \frac{1}{3!}\sum_R \alpha(R)\,e(Q(R)) = 52$$

TABLE 2

Calculation of the number of phenograms with four allelomorphs

R	Q	$e(Q)$	$\alpha(R)$	$\alpha(R)\,e(Q)$
(1^4)	(1^{10})	115975	1	115975
(21^2)	(2^21^6)	2119	6	12714
(2^2)	(2^41^2)	999	3	2997
(31)	(3^21)	73	8	584
(4)	(4^22)	55	6	330

$$n_4 = \frac{1}{4!}\sum_R \alpha(R)\,e(Q(R)) = 5525$$

TABLE 3

Calculation of the number of phenograms with five allelomorphs

R	Q	$e(Q)$	$\alpha(R)$	$\alpha(R)\,e(Q)$
(1^5)	(1^{15})	1382,958545	1	1382,958545
(21^3)	(2^41^7)	1,748249	10	17,482490
(2^21)	(2^61^3)	214157	15	3,212355
(31^2)	(3^41^3)	4622	20	92440
(32)	(63^221)	386	20	7720
(41)	(4^321)	783	30	23490
(5)	(5^3)	70	24	1680

$$n_5 = \frac{1}{5!}\sum_R \alpha(R)\,e(Q(R)) = 11{,}698156$$

Finally, a cyclic interchange of the three allelomorphs corresponding to the partition (3) will lead to two cyclic interchanges of three genotypes corresponding to the partition (3^2). For example, the cyclic interchange of the three allelomorphs corresponding to (ABC) will lead to the cyclic interchange of the genotypes AA, BB and CC and also of AB, BC and CA.

The numbers of phenograms with four and five allelomorphs are 5525 and 11,698156 respectively, rather less than conjectured by Cotterman (1953), but of the same order of magnitude. The number of phenograms increases very rapidly with the increasing number of allelomorphs, an indication of the great variability inherent in a multi-allelomorphic system.

SUMMARY

A general method is described for the enumeration and classification of the genotype-phenotype relationships possible with a single diploid locus and any number of allelomorphs.

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SELFING UNDER CONDITIONS FAVOURING HETEROZYGOSITY

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1. INTRODUCTION

A locus may be maintained in a heterozygous state experimentally, or by various outbreeding devices such as heterostyly or self-sterility. If one or both of the homozygotes are of lowered viability when compared with the heterozygotes, progress towards homozygosity under any inbreeding programme will be slower. This effect will be enhanced by the unconscious selection of the fitter heterozygotes to continue such a programme. The extreme case is where either or both of the homozygotes are lethal. Bennett (1956) has examined the situation where one of the homozygotes is lethal. Bartlett and Haldane (1935) have examined the progress towards homozygosity of a locus linked to loci maintained permanently heterozygous (*i.e.* both homozygotes are lethal). Hayman and Mather (1953) have discussed various situations where the homozygotes are at a disadvantage.

In the first part of this paper (section 2), using selfing as the inbreeding system, the progress towards homozygosity for a locus linked to a locus maintained permanently heterozygous will be considered, and extended to the case of a locus situated in between two such loci maintained in a permanently heterozygous condition. In the second part of this paper (section 3) selfing of tetrasomics where the homozygotes are at a disadvantage (neglecting linkage) will be considered.

Any delay due to the homozygotes maturing slower, or of unconscious selection for the more vigorous heterozygotes will be ignored. This is a problem in the setting up of inbred lines and will have the effect of slowing the approach of a line to homozygosity. We shall assume that a single mating will continue an inbreeding system.

2. INBREEDING OF A LOCUS LINKED TO LOCI MAINTAINED PERMANENTLY HETEROZYGOUS

Here we shall consider the approach to homozygosity of a locus B linked to locus A with recombination value y, in which only the heterozygotes Aa are viable. This situation was discussed very briefly by Bartlett and Haldane (1935) and it is proposed to extend it here.

There are four possible genotypes :—AB/ab, AB/aB, Ab/ab and

Ab/aB. Denoting their initial frequencies by u_0, v_0, w_0 and x_0 respectively, the generation matrix for selfing is :—

	u_0	v_0	w_0	x_0
u_1	$(1-y)^2$			y^2
v_1	$y(1-y)$	1		$y(1-y)$
w_1	$y(1-y)$		1	$y(1-y)$
x_1	y^2			$(1-y)^2$
Column divisor	1	1	1	1

If λ represents the latent roots of this matrix, an equation in λ (the characteristic equation) may be obtained by equating the determinant of the λ matrix to zero :—

$$[(1-y)^2-\lambda]^2\,(1-\lambda)^2-(1-\lambda)^2y^4=0$$

This equation yields two roots of unity which are trivial as they represent the homozygous genotypes, and a dominant latent root

$$\lambda_D = 1-2y+2y^2$$

and also a fourth root

$$\lambda = 1-2y.$$

In terms of the recombination value y, λ_D takes the following values (table 1) :—

TABLE 1

y per cent.	λ
0	1
0·1	0·9980
0·5	0·9901
1·0	0·9802
5·0	0·9050
10·0	0·8200
25·0	0·6250
50·0	0·5000

For two closely linked loci, locus *B* will approach homozygosity very slowly. For a single gene where one homozygote is lethal, $\lambda_D = \frac{2}{3}$ (Bennett, 1956). In the situation discussed here $\lambda_D \geqslant \frac{2}{3}$ for $y \leqslant 21{\cdot}13$ per cent. As $y \to 50$ per cent., $\lambda_D \to \frac{1}{2}$ which is the dominant latent root obtained when a normal locus, say *B*, unaffected by the presence of *A* is inbred by selfing.

Table 1 is only true if the homozygotes and heterozygotes at the *B* locus are equally viable. If the homozygotes are less viable, λ_D will be somewhat greater, and progress towards homozygosity retarded. Considering *B* only, and giving viability factors *m* and *n* to *BB* and

bb respectively, and to *Bb* a factor of unity, the generation matrix for selfing is :—

	BB	*Bb*	*bb*
BB	*m*		*m*
Bb		*n*	*n*
bb			2
Column divisors	*m*	*n*	*m*+*n*+2

The characteristic equation is :

$$(m-m\lambda)(n-n\lambda)[2-(2+m+n)\lambda] = 0$$

yielding a latent root of unity and the dominant latent root

$$\lambda_D = \frac{2}{2+m+n}$$

Provided that both homozygotes are not lethal, $\lambda_D < 1$ and there will be some progress towards homozygosity. If $m = n = 1$, $\lambda_D = \frac{1}{2}$ which is the latent root obtained from selfing a disomic without any complications.

Returning to the case of *B* linked to *A* which is permanently heterozygous, we give *BB*, *bb*, and *Bb* viabilities of *m*, *n*, and 1 respectively. The generation matrix will become :—

	u_0	v_0	w_0	x_0
u_1	$(1-y)^2$	...	...	y^2
v_1	$y(1-y)m$	m	...	$y(1-y)m$
w_1	$y(1-y)n$	...	n	$y(1-y)n$
x_1	y^2	...	...	$(1-y)^2$
Column divisors	$1-2y+2y^2+y(1-y)(m+n)$	m	n	$1-2y+2y^2+y(1-y)(m+n)$

The latent roots are :—

$$\lambda = 1$$

$$\lambda = \frac{1-2y}{1-2y+2y^2+y(1-y)(m+n)}$$

and

$$\lambda_D = \frac{1-2y+2y^2}{1-2y+2y^2+y(1-y)(m+n)}$$

The last two roots reduce to those given for the case of locus *B* free from viability disturbance when $m = n = 1$. When *m*, $n < 1$, λ_D will increase to a limit of unity at $m = n = 0$. Poor viability of *bb* and *BB* will result in slower progress to homozygosity. If selection

for the more vigorous heterozygotes, or difficulties in maintaining the inbreeding programme are taken into account, progress will be reduced more.

An application of this system is found in *Œnothera* where some species are permanently heterozygous for a series of interchanges (Darlington, 1931 ; Catcheside, 1940). In these species crossing-over is suppressed in the differential segments of the interchanges and different mutations accumulate. Hence the segments no longer correspond and two kinds of gamete are formed. When similar gametes unite the zygote is lethal, due to recessive lethal factors. Normal crossing-over occurs in the pairing segments in the distal regions of chromosome arms. The preceding analysis would apply to these regions, although some rare cross-overs occur in the differential segments.

Bartlett and Haldane (1935) have considered in some detail the problem of sib-mating which will not be discussed here. The result is similar to that for selfing. The dominant latent root drops from unity at complete linkage to 0·8090 at independence, this being the root for ordinary sib-mating.

A more complex case in which there are two linked loci *A* and *C*, with *AA*, *aa*, *CC*, and *cc* lethal, will now be considered. It is required to find the progress towards homozygosity of a locus *B* situated between *A* and *C*. Let p represent no recombination in segments *AB* and *BC*, q represent recombination in segment *AB* but not *BC*, r represent recombination in segment *BC* but not *AB*, and s represent simultaneous recombination in both segments such that

$$p+q+r+s = 1.$$

There are four genotypes with *B* heterozygous.

u_0	*ABC/abc*
v_0	*aBC/Abc*
w_0	*ABc/abC*
x_0	*AbC/aBc*

The generation matrix is :—

	u_0	v_0	w_0	x_0	
u_1	p^2	r^2	q^2	s^2	$\div (p+s)^2+(q-r)^2$
v_1	r^2	p^2	s^2	q^2	
w_1	q^2	s^2	p^2	r^2	
x_1	s^2	q^2	r^2	p^2	

It will be noted that this matrix is a Latin square of the system

A	*B*	*C*	*D*
B	*A*	*D*	*C*
C	*D*	*A*	*B*
D	*C*	*B*	*A*

The determinant of such a matrix is :—

$$(A+B+C+D)(A-B+C-D)(A+B-C-D)(A-B-C+D).$$

Equating the determinant of the λ-matrix to zero we obtain the dominant latent root :—

$$\lambda_1 = \frac{p^2+q^2+r^2+s^2}{(p+s)^2+(q+r)^2} = 1 - \frac{2(ps+qr)}{(p+s)^2+(q+r)^2}$$

and the other three roots :—

$$\lambda_2 = 1 - \frac{2(q^2+s^2+ps+qr)}{(p+s)^2+(q+r)^2}$$

$$\lambda_3 = 1 - \frac{2(r^2+s^2+ps+qr)}{(p+s)^2+(q+r)^2}$$

$$\lambda_4 = 1 - \frac{2(q^2+r^2+ps+qr)}{(p+s)^2+(q+r)^2}$$

It is obvious that $\lambda_1 > \lambda_2, \lambda_3, \lambda_4$ for all p, q, r and s. For a short segment where q and r are small and s is close to zero, λ_1 will be very close to unity. This somewhat hypothetical system can be regarded as the severest possible case of inbreeding where homozygotes AA, aa, CC and cc are of very much poorer viability than the heterozygote.

An application of this type of argument is found when heterozygous inversions are considered. In both paracentric and pericentric inversions, the only surviving gametes are the non cross-over and double cross-over gametes. All single cross-over gametes are lost as they are acentric or dicentric in paracentric inversions, and duplication-deficient in pericentric inversions. Thus we obtain the following generation matrix for the surviving gametes :—

	u_0	v_0	w_0	x_0	
u_1	p^2	...	...	s^2	$\div (p+s)^2$
v_1	...	p^2	s^2	...	
w_1	...	s^2	p^2	...	
x_1	s^2	...	...	p^2	

The dominant latent root is :—

$$\lambda_D = 1 - \frac{2ps}{(p+s)^2}$$

where $p+s$ is unity as $q = r = 0$. Hence $\lambda_D = 1-2ps = 1-2s$ when s is small. Within a short inversion, a heterozygous locus will approach homozygosity very slowly. As an example, let $p = 0{\cdot}60$, $q = 0{\cdot}18$, $r = 0{\cdot}18$ and $s = 0{\cdot}04$, the sum adding to unity. The value for s is rather large for the values of q and r considered. In a heterozygous inversion $q = r = 0$ and $p+s = 1$. Thus p will be $\frac{0{\cdot}60}{0{\cdot}64} = 0{\cdot}9375$ and $s = \frac{0{\cdot}04}{0{\cdot}64} = 0{\cdot}0625$ which gives $\lambda_D = 0{\cdot}8828$.

This is a segment of considerable length and progress towards homozygosity is slow. For $p = 0{\cdot}90$, $q = 0{\cdot}05$, $r = 0{\cdot}047$ and $s = 0{\cdot}003$ which is a fairly realistic situation, we obtain $\lambda_D = 0{\cdot}9934$. Hence for a short inversion heterozygote progress towards homozygosity is virtually non-existent. It is known that inversions preserve favourable gene complexes. This analysis confirms this, for even in the most severe form of inbreeding, namely selfing, little progress towards homozygosity is made for heterozygous inversions of short or medium length. Long inversions, which are occasionally found, where most of the chromosome is inverted, will not, however, preserve favourable gene complexes so readily. This analysis neglects any inhibition of crossing-over near the break points which would reduce progress to homozygosity even more.

3. TETRASOMICS

Selfing of tetrasomics in which homozygotes are at a disadvantage, or lethal, will now be considered. Linkage and double reduction will be ignored. Firstly, a factor A will be considered in which the nulliplex genotype a_4 is lethal and the other four genotypes A_4, A_3a, A_2a_2 and Aa_3 are normal. The generation matrix is :—

	u_0 (A_3)	v_0 (A_3a)	w_0 (A_2a_2)	x_0 (Aa_3)
u_1	1	1	1	...
v_1	...	2	1	...
w_1	...	1	18	1
x_1	...	...	1	2
Column divisors	1	4	35	3

The characteristic equation is :

$$(1-\lambda)(210\lambda^3-353\lambda^2+168\lambda-20) = 0 \quad . \quad . \quad (1)$$

which yields four latent roots :

1
0·932,717 the dominant root
0·568,683
and 0·179,552.

To find the principal component of frequency P the coefficients of a, b, c in

$$P = av+bw+cx$$

are needed, such that $P^1 = \lambda P$ where P^1 represents the next generation. Substituting the value of λ_D in the λ-matrix and treating the columns

of the matrix as linear functions of the coefficients a, b, c we obtain the principal component

$$P = v + 1{\cdot}730{,}869\, w + 2{\cdot}168{,}596\, x$$

the coefficients of v, w and x being the relative complexities (Fisher, 1949).

The limiting frequencies are found by treating the rows of the λ-matrix as equations in v, w and x. We obtain :

$$\begin{aligned} v &= 0{\cdot}221{,}259 \\ w &= 0{\cdot}418{,}874 \\ x &= 0{\cdot}359{,}866 \end{aligned}$$

the sum being unity.

Substituting these limiting frequencies in the linear function P gives the average relative complexity $= 1{\cdot}726{,}681$. Dividing P by this value will give the three absolute complexities :

$$\begin{aligned} V &= 0{\cdot}579{,}146 \\ W &= 1{\cdot}002{,}426 \\ X &= 1{\cdot}255{,}933 \end{aligned}$$

As a check, the sum of the products of the frequencies with the absolute complexities should be unity (Fisher, 1949), *i.e.*

$$vV + wW + xX = 1.$$

Progress towards homozygosity for any mating type depends upon its absolute complexity. Comparing the three heterogenic mating types with an absolute complexity of unity as standard (Fisher, 1949) we find that u is 7·842 generations ahead of the standard, and v and w are 0·034 and 3·271 generations behind the standard of unity respectively. Hence mating type Aa_3 is in the worst condition and mating type A_3a in the best when considering their approach to homozygosity.

This system may be compared with selfing of tetrasomics without disturbance for which $\lambda_D = \frac{5}{6}$ (neglecting double reduction). One generation of selfing of normal tetrasomics is equivalent to

$$(\log_e 0{\cdot}8333)/(\log_e 0{\cdot}9327) = 2{\cdot}618$$

generations of selfing of tetrasomics with a_4 lethal. The presence of this lethal genotype therefore retards progress towards homozygosity considerably. For disomics Bennett (1956) gives $\lambda_D = \frac{2}{3}$ for the situation in which one of the two homozygotes is lethal. One generation of selfing of normal disomics is equivalent to

$$(\log_e 0{\cdot}5000)/(\log_e 0{\cdot}6667) = 1{\cdot}709$$

generations of selfing of disomics with one homozygote lethal. Hence, lethality of one of the homozygotes in tetrasomics reduces the progress of inbreeding more than for disomics.

A second situation in tetrasomics where homozygotes are at a

disadvantage will now be considered. Let the duplex genotypes have a viability of unity, simplex and triplex $1-t$, and nulliplex and quadriplex $1-2t$. The generation matrix is :—

	A_4	A_3a	A_2a_2	Aa_3	a_4
A_4	$1-2t$	$1-2t$	$1-2t$	...	...
A_3a	...	$2(1-t)$	$8(1-t)$	...	...
A_2a_2	...	1	18	1	...
Aa_3	...	...	$8(1-t)$	$2(1-t)$	...
a_4	...	...	$1-2t$	$1-2t$	$1-2t$
Column divisor	$1-2t$	$4-4t$	$36-20t$	$4-4t$	$1-2t$

Isomorphic pairs (A_4, a_4) and (A_3a, Aa_3) may be combined to obtain :

	A_4	A_3a	A_2a_2
A_4	$1-2t$	$1-2t$	$1-2t$
A_3a	...	$2(1-t)$	$8(1-t)$
A_2a_2	...	1	9
Column divisor	$1-2t$	$4-4t$	$18-10t$

A latent root $\lambda = 1$ is obtained, and two roots from

$$\lambda^2(36-20t)-\lambda(36-10t)+5 = 0 \quad . \quad . \quad . \quad (2)$$

In terms of t, the dominant latent root takes the following values.

t	λ
0	0·8333
0·01	0·8357
0·05	0·8453
0·1	0·8580
0·25	0·9018
0·5	1·0000

At $t = 0$ the characteristic equation gives the two roots $\frac{1}{6}$ and $\frac{5}{6}$, the values obtained for selfing without disturbance. At $t = 0{\cdot}5$ the two roots are 1 and $\frac{5}{26}$ at which stage the homozygotes A_4 and a_4 will be lethal, and progress towards homozygosity halted. Various other systems where heterozygotes are at an advantage can be postulated, but the results should conform in a general way to those given.

This type of problem may be generalised for all cases of variable viabilities and lethality (zero viability). Let the viabilities of A_4,

A_3a, A_2a_2, Aa_3 and a_4 be a, b, c, d and e respectively. Then the generation matrix becomes :

	A_4	A_3a	A_2a_2	Aa_3	a_4
A_4	■	a	■	...	...
A_3a	...	$2b$	$8b$	...	...
A_2a_2	...	c	$18c$	c	...
Aa_3	...	...	$8d$	$2d$	...
a_4	...	...	e	e	e
Column divisor	a	$a+2b+c$	$a+8b+18c+8d+e$	$c+2d+e$	e

This yields two latent roots of unity and three from the equation :

$$[2b-\lambda(a+2b+c)][18c-\lambda(a+8b+18c+8d+e)]$$
$$[2d-\lambda(1+2d+e)]-8cd[2b-\lambda(a+2b+c)]$$
$$-8bc[2d-\lambda(c+2d+e)] = 0.$$

If we let $a = e = 1-2t$, $b = d = 1-t$ and $c = 1$ which is the viability problem discussed above, the root $\lambda = \frac{1}{2}$ is obtained, and the equation

$$\lambda^2(36-20t)-\lambda(36-10t)+5 = 0$$

from which λ_D is obtained. This is the equation (2) already given for this problem.

Similarly, considering the situation where a_4 is lethal, we can let $a = b = c = d = 1$ and $e = 1$. The general equation reduces to

$$(1-\lambda)(210\,\lambda^3-353\,\lambda^2+168\,\lambda-20) = 0$$

which is the equation (1) given for this problem.

In conclusion, as for disomics, progress towards homozygosity will be slower if the heterozygotes are at an advantage. In the severest case, where one or both of the homozygotes are lethal for inbreeding progress will be very slow, or even halted.

4. DISCUSSION

Although some of the examples selected may appear to be of little direct application, it is evident from this analysis, and from those of other authors, that the maintenance of heterozygosity in any inbreeding system (selfing is used here for simplicity) will slow down the rate of approach to homozygosity. Where homozygotes are of poor viability, the progress towards homozygosity will be retarded, this being manifest by a higher value of λ_D than normal. The effect of enforced heterozygosis does not only affect the locus concerned, but also affects neighbouring loci dependent upon the recombination value with the locus at which homozygotes are disadvantageous. In the extreme case, where both homozygotes are lethal, as in some species of *Œnothera*,

λ_D for that locus is unity, and we can only study the approach to homozygosity of a linked locus.

Progress towards homozygosity within a short inversion heterozygote is almost negligible, and here we have enforced heterozygosity of the severest form. The stability of the gene complex within an inversion allows favourable gene complexes to be preserved. In some species of *Drosophila*, notably *D. willistoni*, heterozygous inversions are particularly common (Dobzhansky, Burla and Da Cunha, 1950). The frequency of specific inversion heterozygotes appears to vary according to environment. There are regular seasonal cycles in the frequency of various inversions, these cycles being repeated annually (Dobzhansky, 1943). Such studies show beyond doubt that inversion heterozygotes are of advantage, and the present analysis shows the difficulty of inducing any change within the inversion heterozygote for the severest possible form of inbreeding. Recombination is also restricted to a certain extent outside an inversion heterozygote (Dobzhansky and Epling, 1948), so preserving additional gene complexes.

It is likely that favourable gene complexes could be preserved to a certain degree if such a complex were situated between two relatively closely linked loci for which the heterozygotes are at a considerable advantage compared with homozygotes. The extreme case, where homozygotes are lethal, is analysed in this paper. In this extreme case λ_D is close to unity for short segments and progress towards homozygosity is very slow.

For the selfing of tetrasomics, λ_D is close to unity for loci where the homozygotes are near to lethality. Such a locus will enforce a certain degree of heterozygosity on linked loci, the magnitude of the enforced heterozygosis decreasing with distance. Progress towards homozygosity for tetrasomics is generally slower than for disomics. Lethal genes in tetrasomics reduce the approach to homozygosity considerably and enforce some degree of heterozygosity. A measure of the rate of elimination of the lethal gene, in the situation where a_4 is lethal is given by $-\log_e \lambda_D = 0{\cdot}070$ compared with $-\log_e(\frac{2}{3}) = 0{\cdot}405$ (Bennett, 1950) for disomics with one homozygote lethal. Tetraploids therefore shelter lethal genes, these in turn cause enforced heterozygosis.

Generally, therefore, the presence of lethals, or of deleterious homozygotes will slow down any theoretical inbreeding system in practice, and will result in enforced heterozygosity. Furthermore, this applies to linked but unaffected loci, the extreme case being a locus situated in an inversion heterozygote.

Throughout this discussion, delay due to inadequate or slowly maturing progeny has been ignored. It is also assumed that there is no unconscious selection for the more vigorous heterozygotes. If these considerations had been taken into account, the progress of inbreeding would be slower.

5. SUMMARY

The progress towards homozygosity is considered for situations where homozygotes are at a disadvantage or lethal. Selfing, although impossible in some organisms, provides a basis for comparison and has the advantage of relatively simple algebra. Matrix methods are used in the calculations.

Where homozygotes are of poor viability the approach to homozygosity is slower than for homozygotes and heterozygotes of the same viability. Loci linked to those in which heterozygosis is favoured approach homozygosity slower than if they were independent, the extreme case being the heterozygous inversion.

Acknowledgment.—I would like to thank Dr A. R. G. Owen for many helpful discussions.

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REVIEWS

GENETICS IN PLANT BREEDING. Brookhaven Symposia in Biology, No. 9. Brookhaven National Laboratory, New York. Pp. I-ix, 1-236. $1.25.

This report, which is the ninth in the series of symposia arranged by the Brookhaven National Laboratory, introduces most of the important current research on plant breeding being done in the U.S.A. It includes sessions on aspects of cytology, biometrics, reproduction and mutation which are basic to the methods of plant breeding. The subject matter emphasises the fundamental approach and preserves a fine balance between the many different techniques and mental approaches that have a place in the planned improvement of crops. As a result of limiting the number of participants, the contributions achieve a high standard throughout and the lengthy discussions which are printed in full testify to a degree of interest and stimulus which is quite rare. Apart from a few irritating statements like " Evolution proceeds by producing new species " on p. 123 and a tiring discussion on whether induced mutations are " progressive " or " retrogressive " on p. 174 the report is very readable and well edited. The contributions are well documented and provide valuable points of reference.

Two contributions are outstanding. They are E. R. Sears's account of the successful transfer of leaf rust resistance from *Ægilops umbellata* to wheat, and J. R. Harlan's treatment of the utilisation of natural variability in cultivated plants. In an elegant piece of cytological research Sears achieved the transfer of rust resistance from a related genus through the use of an amphidiploid of *Triticum dicoccoides* and *A. umbellata* as a " bridge " to *T. æstivum*. An intercalary segment bearing resistance, but lacking deleterious characters was incorporated in the wheat genome, producing an entirely new genotype whose morphological expression was indistinguishable from the wheat parent. The consequence of this single transfer will probably be felt ultimately in all the wheat breeding programmes of the world and its importance to the economy of the wheat producing areas must be obvious. This example of " chromosome building " using widely related species represents the most deliberate attempt to date at directing the evolution of chromosomes in the service of man, and is one that must have immense possibilities for the future.

Harlan's contribution deserves special mention because it demonstrates the achievements of utilising the world's reservoir of variability in crop plants. The " composite cross " method as applied to barley is described as a means of exposing new collections to recombination and selection. One such cross involving 28 parents and grown continuously for 29 generations yielded valuable information on the most profitable sources of variation and showed that these need not coincide with centres of maximum diversity. That this is what one would expect is no excuse for the principle to have been overlooked in previous discussions on this subject. In addition the method has achieved practical breeding success, and segregating populations subjected to over twenty years of natural selection have yielded isolates which exceed the standard variety by as much as 56 per cent.

Four contributions deal with the production of economically valuable mutations by the use of radiations. Although much of the work reported on this subject is not convincing, the demonstrations by D. Lewis of the disruption of the breeding system in self-incompatible species, and by C. F. Konzak of the induction of resistance to Victoria blight in susceptible oat varieties, illustrate that mutagenic agents have definite possibilities in plant breeding. The success of both attempts at achieving valuable mutations depend on their well defined genetic objective and on an efficient screening technique for the isolation of the mutant genotype.

The symposium achieved what it set out to do, namely to highlight recent developments in genetic research which are of potential value to plant breeding. These are impressive and one hopes that readers will find it no longer necessary to cite " hybrid corn " as the sole contribution of genetics to agriculture. WATKIN WILLIAMS.

CHROMOSOME ATLAS OF FLOWERING PLANTS. 2nd Ed. By C. D. Darlington and A. P. Wylie. London : George Allen and Unwin. 1955. Pp. 519+xix. 60s.

CHROMOSOME BOTANY. By C. D. Darlington. London : George Allen and Unwin. 1956. Pp. 186+xii. 16s.

The second edition of the invaluable *Chromosome Atlas of Cultivated Plants* has been widened in scope to include all flowering plants, hence the change of title. It covers the chromosome numbers of nearly double the number of plants represented in its predecessor. The genera with uniform chromosome numbers have been abbreviated, the variable ones being given in detail, including reference to the presence of B chromosomes. The families, numbered and in the order of Hutchinson's *Families of Flowering Plants*, are divided into 25 groups of orders, each being preceded by an illustration of the chromosomes of a member of the group. The rich data convey to systematists the value of chromosome numbers for classification, suggest to plant breeders the breeding system and the limits within which interspecific crosses might be attempted, and show cytologists what has been done and what remains to be done.

Issue may be taken with the reasons given for omitting authors' names from the species listed. These are part of the name of the abstract taxonomic concept embodied in a diagnosis. Truly the type specimen's chromosome number is only conjecturally that of the specimens observed, but presumably these resemble, *i.e.* conform to the diagnosis of, the taxonomic species whose name is used in the Atlas. We surely need to know in whose specific taxonomic sense the name is used.

The interpretation of the material in the Atlas, formerly in its introduction, is incorporated in the companion volume on *Chromosome Botany*, which discusses the laws of chromosome variation. It is a stimulating book, which treats the chromosomes of plants as broadly as possible, consistent with the omission of the reciprocal connection with formal genetics. The intricacies are avoided partly by a descriptive treatment and partly by pointing to problems, rather than expounding their solutions. This method of presentation is undoubtedly well suited to students of botany, accustomed to descriptive analysis, who should study this book closely. Nevertheless the description is sometimes so couched as to suggest that no problems

remain, as in the chemical structure of chromosomes or the mechanism of crossing-over.

The material is presented in seven chapters (1) structure of chromosomes and their behaviour in division, structural and numerical change and the nature and behaviour of B chromosomes, (2) systematics, the kinds of natural species, their modes of reproduction and mechanisms of variation, (3) the ecology and geography of cytological variation, (4) chromosome number and individuality and their directions and rates of change as a function of time, (5) the evolution of cultivated plants, embodying the introduction to the first edition of the Atlas, (6) the modes of evolution of cultivated ornamental plants, ranging from mutation in the sweet pea, through aneuploidy in the hyacinth to the complex hybridisations to which we owe our roses, (7) lessons.

A few errors should be noted. The statement (p. 24) that pollen grains with more B chromosomes accomplish fertilisation more quickly, is presumably founded on the competition between male nuclei with differing numbers of B chromosomes, but this competition occurs between nuclei in the same pollen grain. *Lilium perenne* (p. 100) is *Lolium p.* The Palestinian form of *Trifolium subterraneum* (p. 101) is morphologically distinct and recognised as var. *telavivensis* in Post's Flora.

The principles of classification are sketched somewhat tendentiously in Chapter 2. No clear distinction is drawn between natural and taxonomic species, and this philosophic omission is probably responsible for much of the impatience of the experimenter with classical taxonomy. Taxonomic species are abstract concepts arising out of the attempts to describe the natural species, assumed to exist in nature. The latter are essentially mating groups, but in erecting taxonomic species the taxonomist necessarily relies mainly upon practical rules based upon differences of form and spatial distribution. Cytology serves to show the existence of distinct mating groups where previously they were unsuspected.

These defects do not detract from the excellence of this book which is an original and distinctive contribution to biological knowledge. Its principles indeed are applicable, as we would expect and as E. B. Ford shows in an appendix, to animals. D. G. CATCHESIDE.

A SYMPOSIUM ON THE CHEMICAL BASIS OF HEREDITY. Edited by William D. McElroy and Bentley Glass. Johns Hopkins University Press and Oxford University Press. 1957. Pp. xi+848. £5.

This is a report of twenty-seven papers connected with the chemistry of chromosomes, genes or viruses or the mechanics of their propagation or recombination. There are discussions of sixteen of the papers which make the work as a whole more disconnected, and an 80-page summary by Dr Bentley Glass which attempts to make it more connected. Some of the papers treat their subject in a general way with copious bibliographies, others in unrelieved and unreferenced detail. The field is somewhat too wide for this heterogeneous method of treatment and the value of the whole work lies in certain outstandingly useful contributions on bacterial and phage genetics. There is a useful index. C. D. D.

ATOMIC ENERGY IN MEDICINE. By K. E. Halnan. Butterworth's Scientific Publications. 1957. Pp. ix+157. 15s.

This book deals in a simple introductory way with the uses of radiation, especially by way of radioactive isotopes, in medical diagnosis and treatment. There is a chapter on the hazards of radiation, including the genetic hazards, and a short bibliography. C. D. D.

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HUMAN BLOOD GROUPS AND INHERITANCE. By Sylvia D. Lawler and L. J. Lawler. 2nd Ed. William Heinemann. 1957. Pp. viii+103. 7s. 6d.

PRINCIPLES OF IMMUNOLOGY. By John E. Cushing and Dan H. Campbell. McGraw-Hill Book Company. 1957. Pp. ix+344. $6.50.

ELEMENTS OF GENETICS. By Edward C. Colin. 3rd Ed. McGraw-Hill Book Company. 1956. Pp. xv+498. 43s.

GENETICS, THE MODERN SCIENCE OF HEREDITY. By Edward O. Dodson. W. B. Saunders Company. 1956. Pp. viii+329. 45s. 6d.

NATÜRLICHE UND KÜNSTLICHE ERBÄNDERUNGEN. By Hans Marquardt. Rowohlt Verlag. 1957. Pp. 177.

CHROMOSOMES, SEX-CELLS AND EVOLUTION IN A MAMMAL. By Phillip V. Tobias. Percy Lund Humphries. 1956. Pp. xx+420. 60s.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND TWENTY-FOURTH MEETING of the Society, held on 18th and 19th JULY 1957, at the JOHN INNES HORTICULTURAL INSTITUTION, BAYFORDBURY, HERTFORD

ABNORMAL TETRADS IN *COPRINUS LAGOPUS*

P. R. DAY

John Innes Horticultural Institution, Bayfordbury, Hertford

Two wild type monocaryon stocks when intercrossed produce normal or abnormal fruit bodies according to the direction of the cross. The abnormal fruit bodies have pale gills since under 10 per cent. of the basidia produce basidiospores. A high proportion of the " tetrads " produced are abnormal having from two to six basidiospores, many of which are inviable. Meiosis is irregular, most basidia are arrested at metaphase I. The stocks were isolated from the wild in 1952 and have since been maintained under mineral oil. Originally both crosses were normal. The abnormal behaviour was first noted in January 1957. When those stocks have been subcultured at 28° C. several times and are then intercrossed, the reciprocal differences tend to disappear and both crosses produce abnormal fruit bodies. Normal sectors are present in some of these fruit bodies. The facts suggest that the abnormality is determined by the cytoplasm and is a result of ageing.

ABNORMAL GAMETOGENESIS AND EMBRYO ABORTION IN *PYRUS COMMUNIS*

G. J. DOWRICK

John Innes Horticultural Institution, Bayfordbury, Hertford

Sexual reproduction in higher plants may be prevented by a number of mechanisms which can act either before or after fertilisation. In the diploid pear variety Beurré Bedford ($2n = 34$) sterility is related to genetic unbalance of the developing embryo.

Young pollen grains are quadrinucleate as a result of the failure of cell wall formation following meiosis. At the first pollen grain mitosis fusion of spindles may occur. In 80 per cent. of grains all four spindles fuse and give rise to mature pollen grains with a single generative nucleus. In the remainder, one or more nuclei remain free and these form mature pollen grains with a variable number of haploid, diploid and triploid nuclei. The amount of spindle fusion is related to gradients passing across the anther loculus and therefore across individual pollen grains.

Macrosporogenesis is normal in Beurré Bedford. The progeny which are formed when it is used as a female parent have the expected chromosome numbers.

When it is used as male parent, however, and crossed with diploid and tetraploid varieties it yields a preponderance of diploid and tetraploid progeny respectively. Most embryos whose chromosome number is not the same as that of the female parent abort before reaching maturity. It is postulated that this breakdown is due to a dosage controlled embryo lethal mechanism.

BREEDING BEHAVIOUR OF SUBSEXUAL *RUBUS* SPECIES

G. M. HASKELL

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Most wild and cultivated blackberries are polyploid and pseudogamous apomicts. Selection in a large population for prickle intensities and earliness has resulted in extending the range of variation. The highest prickle line showed correlated effects in other characters, *e.g.* shorter internodes, more rounded leaves and poor fertility ; other lines were normal. This behaviour is consistent with the occurrence of meiosis and recombination in *diplosporous* apomictic species.

Sexual species-hybrids in *Rubus* like veitchberry and loganberry (*idaeobatus* × *eubatus*) breed more or less true through autosyndesis, but some variation can be released by recombination. Apomixis in true-breeding *eubati* disguises their hybrid nature, which cannot always be recognised taxonomically, owing to the almost complete dominance of one species. Hybridity is broken down when the occasional sexual seedling is produced, or by release of extreme recombinant types. These true sexual hybrids or selfs may show disruptive effects due to unbalance of the new genetic recombinations. The amount of subsexual variation that an apomict can release will depend on the relative frequencies of apospory and diplospory, its original hybridity and the extent of autosyndesis.

The genetic constitution of the male gamete has a considerable influence on vigour and seedling survival in pseudogamous apomicts ; the occurrence of two or more apomictic species in the same habitat may, therefore, have symbiotic value. Progeny from self-pollinating *R. laciniatus* is weaker than when foreign pollen is used. This effect may last two or more years, for the " cross-pollinated " progeny flower a year before the " self-pollinated ". It is suggested that pseudogamy in *Rubus* is at the " twilight " evolutionary stage between complete sexuality (*i.e.* fertilisation of egg and secondary nucleus) and complete non-sexuality. The genetic constitution of the sexually formed endosperm affects the vigour of the apomictic embryo and seedling.

GENETIC SYSTEMS OF THE GENUS *STREPTOCARPUS*

W. J. C. LAWRENCE

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THE BIOGENESIS OF THE FLAVONOID PLANT PIGMENTS

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The distribution of the flavonoids, particularly the anthocyanins and flavonols, has been studied in a variety of plant material using modern techniques. The pigments present in series of colour mutants of several cultivated species have been examined in detail. The flavonoids of related wild species have been surveyed more briefly.

The results provide information concerning the biogenesis of these pigments. Anthocyanidins and flavonols with the same hydroxylation pattern are frequently found to occur together and their relationship has become clearer. Both types of pigment are produced by a similar metabolic pathway. However, in the final elaboration of the anthocyanidin pigments by genes controlling methylation, glycosidation and acylation, the flavonols are not involved. There is also evidence of competition for precursor between the pigments of both kinds at the three different levels of hydroxylation. This situation is further complicated by the fact that the genes controlling pigment production may exert their effects differently in the foliar and floral parts.

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